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THE EFFECT OF NARCOSIS ON ELECTROTONUS

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From the Rice Institute, Houston, Texas

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Pflüger showed that during the passage of a galvanic current through a nerve the physiological condition of the nerve in the neighborhood of the poles is changed. Currents—"electrotonic currents"—are set up near both poles. In the region around the anode the irritability, as measured by the threshold for stimulation, and the conductivity, as measured by the speed of the nerve impulse, are decreased; in the region around the cathode, provided the current is not too strong, the irritability and the conductivity are increased. If the current is long continued, however, the cathodal hyper-irritability and hyper-conductivity are lessened and may even change to sub-normal values; such sub-normal values appear very quickly or at once if the polarizing current is very strong.

The above effects are those observed in non-narcotized nerve. It should also be of interest to examine what effects are produced by the electric current in nerve that has been narcotized.

In regard to the electrotonic currents, Waller found that these are set up by a polarizing current even in a "completely" narcotized nerve, although not in a dead nerve, and this is in accord with Sosnowsky's statement that medullated rabbit nerves continue to show electrotonic currents for several days after they have lost their ability to conduct an impulse.

The question then arises: How does narcosis affect the changes in irritability and conductivity that are brought about by a polarizing current? And this in turn raises two questions. What is the nature of electrotonic changes in irritability and conductivity in the "par-

tially" narcotized portion of nerve itself, that is, in the region of decrement? And, does a "completely" narcotized stretch of nerve block the spread of the electrotonic change in irritability and conductivity to regions beyond in the same sense at it blocks the spread of the nerve impulse? The answers to these questions are furnished by the following experiments.

Let us first consider electrotonic changes of *conductivity* in a region of decrement.

The sciatic nerve of a frog muscle-nerve preparation was passed through small holes in the sides of a narcotizing chamber (an ebonite chamber, about 1 cm. in diameter) (fig. 1). The holes were plugged carefully with a thick paste of kaolin moistened with Ringer's solution. By this arrangement the portion of the nerve in the chamber was sep-

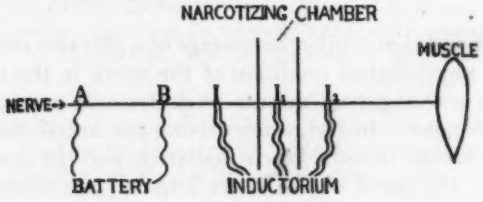

Fig. 1

arated from the rest of the nerve, so that it alone might be exposed to the action of a narcotic, e.g., ether fumes. *A* and *B*, both proximal to the chamber, were non-polarizable boot electrodes of the polarizing circuit; *A*, the more proximal electrode, being the anode, and *B*, the more distal electrode, the cathode. *B* was placed in close enough proximity to the narcotizing chamber for catelectrotonic changes to spread from it over the stretch of nerve contained within. At *I*, between the distal electrode and the narcotizing chamber, were platinum electrodes connected with an inductorium. The whole was placed in a moist chamber. The source of the polarizing current was two ordinary dry cells (about two volts). This strength of current caused increased irritability at the cathode, as measured by the minimal strength of induction shock necessary to cause muscular contraction. The inductorium was now set so that the stimulus was above the threshold. In the following table, which is a protocol of one of the experiments, R signifies muscular contraction; r, no muscular contraction; the first column, I, shows whether or not the induction shock caused muscu-

lar contraction when applied in the absence of the polarizing current; column M shows whether or not muscular contraction occurred at the make of the polarizing current; IC shows the result from the induction shock applied while the polarizing current was passing through the nerve; B shows the result at the break of the polarizing current. The stimuli were applied in cycles in this order and sufficient time was allowed between stimuli for recovery in the muscle. The numbers in the table refer to the successive cycles of stimuli. At the point marked "narcosis started," air bubbled through ether and water was passed

TABLE 1

Narcosis applied to the region subject to catelectrotonus

	t	M	IC	B
1. Without narcosis	R	R	R	R
2. Narcosis started	R	R	R	R
3. Complete narcosis	r	r	r	r
4. "Partial" narcosis	R	R	r	r
5. "Partial" narcosis	R	R	r	r
6. "Partial" narcosis	R	R	r	r
7. "Partial" narcosis	R	R	r	r
8. "Partial" narcosis	R	R	r	r
9. "Partial" narcosis	R	R	r	r
10. "Partial" narcosis	R	R	r	r
11. "Partial" narcosis	R	R	r	r
12. "Partial" narcosis	R	R	r	r
13. "Partial" narcosis	R	R	r	r
14. "Partial" narcosis	R	R	r	r
15. "Partial" narcosis	R	R	r	r
16. "Partial" narcosis	R	R	r	r
17. Recovery complete	R	R	R	R
18. Recovery complete	R	R	R	R

through the narcotizing chamber; at the first point marked "partial narcosis," the ether in the chamber was replaced by air bubbled through water.

1, in table 1, shows that an induction shock (above the threshold) called out muscular response, both without and with the polarizing current acting; it shows also that muscular contraction occurred both at the make and at the break of the polarizing current. This is the condition normally found in the nerve, in the case of a moderate polarizing current, and may be called stage N. Ether fumes were then started through the narcotizing chamber. At 3, all impulses failed to

get through the region of decrement. This may be called stage C, "complete" narcosis. The ether was now displaced by air saturated with water vapor. The portion of the nerve in the chamber now began to regain its conductivity. In this stage of "partial" narcosis (stage P), from 4 to 16 inclusive, induction shocks of the same strength as those used in 1, caused muscular contraction if applied in the absence of the polarizing current, but failed to do so if applied while the polarizing current was passing through the nerve. At the same time muscular response occurred at the make of the polarizing current but not at the break. After still further recovery of conductivity (17 and 18), the polarizing current failed to prevent muscular response to an induction shock; at this time muscular contraction occurred at the break of the polarizing current as well as at the make. Thus the nerve had reëntered stage N. Experiments have shown that stage P, partial narcosis, may be gotten not only when the nerve is coming out of narcosis, but also when it is going into narcosis.

The chief point brought out by this experiment is that—whereas in the non-narcotized region of the nerve catelectrotonus involves an increase of conductivity for the impulse—*in the region of the nerve under partial narcosis catelectrotonus involves a decrease of conductivity; the conductivity change of catelectrotonus thus becomes reversed in sign under narcosis;* for, during the stage called "partial narcosis," impulses are not blocked in the region of decrement so long as the polarizing current is off—contraction regularly following stimulation, but impulses are blocked at the region of decrement during the passage of the polarizing current, and no muscular contraction can be obtained. The reason why contraction occurs at the make but not at the break will be considered later.

The effect of anelectrotonus on conductivity, in a region of decrement, is shown by an experiment similar to that above, but with *A* the cathode and *B* the anode (see fig. 1). The results are shown in table 2.

As in the case of catelectrotonus, three stages are seen as the depth of narcosis increases towards "completeness," they are also seen during recovery: Stage N: Before the exposure of the nerve in the chamber to ether fumes, muscular contraction followed an induction shock, both when the shock was applied in the absence and when it was applied in the presence of the polarizing current (1 in table 2). Stage P: After a certain degree of narcosis was reached (3, 4, 5), the induction shock called out a muscular response if applied in the absence of a polarizing current but failed to do so if applied during the passage

of the polarizing current. At the same time response occurred at the break but not at the make of the polarizing current; this is the reverse of what is gotten in the corresponding stage when the cathode is nearer the chamber. Stage C: All impulses fail to get through. During recovery the opposite sequence of stages was observed.

From this experiment it is evident that anelectrotonus, like catelectrotonus, decreases conductivity in a region of decrement. As anelectrotonus normally decreases conductivity, however, it is evident that the anelectrotonic conductivity change, unlike that of catelectrotonus, is not reversed in sign under narcosis; it summates with narcosis.

Nos. 2 and 9 (table 2) bring out a rather unexpected point. In these cycles of stimuli, muscular contraction did not occur at the make

TABLE 2

Narcosis in region subject to anelectrotonus

	I	M	IC	B
1. Without narcosis	R	R	R	R
2. Narcosis started	R	r	R	R
3. "Partial" narcosis	R	r	r	R
4. "Partial" narcosis	R	r	r	R
5. "Partial" narcosis	R	r	r	R
6. Narcosis "complete"	r	r	r	r
7. "Partial" narcosis	R	r	r	R
8. "Partial" narcosis	R	r	r	R
9. "Partial" narcosis	R	r	R	R
10. Recovery "Complete"	R	R	R	R

of the polarizing current although it did occur when a stimulus was applied during the passage of the polarizing current, and it did occur at the break. Hence the decrease of conductivity in the region of decrement is greater immediately after closing the polarizing current than it is later on during the passage of the current or immediately after the break.

We may now consider why the make and the break of the polarizing current do not have the same effects. For this purpose tables 1 and 2 may be put in the form of Pflüger's law of stimulation by moderate ascending and descending currents. This is shown in table 3. It will be seen here that narcosis causes a modification of Pflüger's law, of such nature that, when there is a region of decrement, the electrotonic effect of a moderate current is similar to the ordinary effect of a very strong current.

In the case of the descending current, the make stimulus, arising at the cathode B, arrives at the region of decrement before catelectrotonic decrease of conductivity appears in that region, so that the impulse gets through. A longer interval of time than that taken by the nerve impulse in traveling from B through the region of decrement must therefore elapse before the full catelectrotonic decrease of conductivity, also originating from B, appears in the region of decrement. The break impulse, arising from the anode A, arrives at the region of decrement before the catelectrotonic decrease of conductivity disappears, so that the impulse is put out.

In the case of the ascending current, on the other hand, by the time the make stimulus, arising at the cathode A, arrives at the region of decrement, anelectrotonic decrease of conductivity, originating from B, has already appeared in that region, so that the impulse is blocked.

TABLE 3

Pflüger's law for moderate currents, as affected by narcosis

	ASCENDING CURRENT (FROM TABLE 2)		DESCENDING CURRENT (FROM TABLE 1)	
	Make	Break	Make	Break
Without a region of decrement	Response	Response	Response	Response
With region of decrement	No response	Response	Response	No response

By the time the break impulse, which arises at the anode B, arrives at the region of decrement, anelectrotonic decrease of conductivity has disappeared, and the impulse gets through.

An idea of the time required for the appearance of anelectrotonus in a distant extra-polar region may be gotten in the following manner. In table 2 it was seen that the make impulse, starting at A (fig. 1), failed to get through when the stretch of nerve in the chamber was partially narcotized. Now, if the time interval which elapses before the appearance of anelectrotonus is greater than that required for a nerve impulse to travel from B to the region of decrement, then it would be possible to get muscular contraction on the make of the polarizing current, as well as on the break, by bringing A sufficiently near B. In fact there would be a position for A such that the time required for the impulse to travel from that point A, through the region of decrement, is equal to the time required for anelectrotonus from B to appear in the region of decrement.

In the following experiment *B* was 2 cm. from the narcotizing chamber. After a region of decrement was established by narcosis, muscular contraction occurred only at the break of the polarizing current, until *A* was brought within 0.5 cm. of *B*, and thus within 2.5 cm. of the narcotizing chamber; now muscular contraction occurred both at the make and at the break of the polarizing current. On moving *A* back further from *B*, a response occurred only at the break. (Five dry cells were used in the polarizing circuit.) Approximately the same results were obtained in six other similar experiments. This shows that the time required for anelectrotonus to appear 2 cm. from the anode, in amounts sufficient to block an impulse in a region of decrement 0.5 cm. long, is equal to the time required for the nerve impulse to travel a distance slightly over 2.5 cm., plus the refractory period of the nerve. In estimating this time it should be remembered that the impulse has to travel through an anelectrotonic stretch of nerve, in which the speed of conduction is considerably reduced.

The time required for the disappearance of anelectrotonus from a region of decrement is much shorter. This is shown in experiments with ascending currents, in which the anode was as near the narcotizing chamber as 0.5 cm.; during the passage of the polarizing current the anelectrotonic effect was sufficient to block the impulses in the region of decrement, yet at the break a response was obtained—the anelectrotonus had disappeared before the impulse had traveled 0.5 cm. It will be observed that this is in marked contrast to the result obtained with catelectrotonus,—in that case, the anode being at A, the impulse started at the break had to travel a considerable distance (4 cm.) before it reached the narcotized region; nevertheless that region was still so affected by the catelectrotonus that the impulse failed to get through.

Further experiments showed that the after-effect of anelectrotonus in a region of decrement is an increase in conductivity. After narcosis has progressed to such a point that impulses from the proximal portion of the nerve are blocked in the region of decrement even when the polarizing current is off, nevertheless they will still go through if they arrive at the region of decrement soon after the breaking of the polarizing current. In an experiment illustrating this effect the anode, which was, as before, in position *B*, was placed at about 1 cm. from the narcotizing chamber; the cathode was placed 4 cm. from the anode. Ether was passed through the chamber until no response was obtained from induction shocks applied between *A* and *B*. The inductorium elec-

trodes were then removed and the galvanic current was closed; no muscular response occurred on the make. On the break of the polarizing current, however, muscular contraction occurred. Soon afterwards "complete" narcosis made its appearance. Tests made during recovery in the narcotized stretch gave the following: first, contraction occurred on the break of the galvanic current, but not from induction shocks, nor on the make of the current. Then there was a time during which muscular response followed induction shock, as well as the break of the polarizing current; but no muscular response occurred at the make. Finally, muscular contraction occurred at the break of the polarizing current, from the induction shock, and at the make. This shows conclusively that a portion of the nerve so deeply narcotized as to block all impulses may be made conductive as an after-effect of anelectrotonus (i. e., at the break of the current). The change from decreased to increased conductivity must take place very quickly after breaking the current.

The electrotonic changes in *irritability* during narcosis may next be considered. Those which occur in the case of catelectrotonus will be dealt with first.

Electrodes connected with an inductorium were placed *inside* the narcotizing chamber (at point I_1, fig. 1), in contact with the nerve. The anode of the polarizing current was at A, the cathode at B, so that the region to be etherized might be subjected to catelectrotonus. At first the irritability of this portion of the nerve was tested without a narcotic. It was found that with the polarizing current on, irritability in this region was increased as measured by the strength of induction shock necessary to cause muscular response. The inductorium was then set at a strength above the threshold necessary for stimulation while the polarizing current was off, and air bubbled through ether was now passed through the narcotizing chamber. As narcosis in the region of decrement gradually progressed, stimuli from the inductorium were sent in alternately without and with the polarizing current on. A typical protocol was as follows:

Results from stimulation within a partially narcotized region, subject to catelectrotonus, narcosis progressively increasing

	POLARIZING CURRENT OFF	POLARIZING CURRENT ON
1	Response	Response
2	Response	Response
3	Response	No response
4	Response	No response
	Stronger induction shock	
5	Response	Response
6	Response	Response

In 3 and 4 an induction shock applied during the passage of the polarizing current did not cause muscular contraction, although in the absence of the polarizing current an induction shock of the same strength did produce a muscular response. This might be due to electrotonic decrease of either irritability or conductivity; that is, the stimulus may have become subminimal and failed to start an impulse, or the impulse, once started, may have been blocked. Nos. 5 and 6 show that the impulses were not blocked, for it required merely a stronger stimulus in order to obtain a muscular response: irritability, therefore, had become decreased under catelectrotonus. *Thus, under narcosis, not only is the conductivity change of catelectrotonus reversed in sign, but also the irritability change.*

Similar experiments showed that anelectrotonus too decreases irritability in the region of decrement. Here again we see that the effects on irritability run parallel with those on conductivity.

Experiments were also undertaken to determine whether electrotonus can "spread" from its pole of origin, through a narcotized region, out into a normal region beyond. For this purpose the inductorium electrodes were placed at I_2 (fig. 1), on the other (distal) side of the narcotizing chamber from the polarizing electrodes A and B. Table 4 (a) shows the effect of anelectrotonus from B on irritability at the point I_2; table 4 (b) shows the effect of catelectrotonus from B on irritability at the same point. In both cases ether was the narcotic used and the portion in the chamber was "completely" narcotized, no muscular contraction resulting when the polarizing current was opened or closed.

It is quite evident here that both anelectrotonus and catelectrotonus are "transmitted" unchanged in nature through the region under narcosis, even though in this region itself they may be reversed in sign and although impulses in this region are blocked.

Experiments using as the narcotic chloral hydrate, magnesium sulphate, ethyl alcohol and zinc sulphate (saturated solution), all gave similar results. In many of these experiments the region in the narcotizing chamber had been so long under narcosis, and had been subjected to such high concentrations of the narcotic, that it must almost

TABLE 4

The spread of electrotonus through a narcotized region

(a) Anelectrotonus: Anode at *B* (fig. 1)

	POLARIZING CURRENT OFF; THRESHOLD STIMULUS FROM INDUCTORIUM	POLARIZING CURRENT ON; STIMULUS OF SAME STRENGTH AS IN FIRST COLUMN
1	Response	No response
2	Response	No response
3	Response	No response
4	Response	No response
	etc	

(b) Catelectrotonus: Cathode at *B*

	POLARIZING CURRENT OFF; STIMULUS FROM INDUCTORIUM JUST BELOW THRESHOLD	POLARIZING CURRENT ON; STIMULUS OF SAME STRENGTH AS IN FIRST COLUMN
1	No response	Response
2	No response	Response
3	No response	Response
4	No response	Response
	etc.	

certainly have been "dead;" later, it failed to recover from the effects of the narcotic and could not be made to conduct an impulse. Nevertheless, electrotonus was transmitted through these regions.

It should be noted, however, that in one experiment, after soaking the portion of the nerve in the narcotizing chamber in zinc sulphate for two hours, electrotonic changes in irritability failed to spread through to the point of stimulation, although they did so for quite a time (thirty minutes) after conductivity had been abolished. Moreover, it was found that if the nerve is cut, instead of having a portion

narcotized, the ends being placed close together, electrotonic changes in irritability started in the free piece of nerve do not get into the portion of the nerve attached to the muscle. Accordingly we must conclude that the processes whereby electrotonus spreads from the polarizing electrode, although not dependent upon properties peculiar to the functioning or the living nerve, nevertheless require some structure or substances other than the grosser physical and chemical characteristics of the nerve.

The following diagram sums up the electrotonic changes occurring *during*[1] the passage of the polarizing current in the three portions of the nerve: *a*, In the non-narcotized proximal portion around the distal polarizing electrode; *b*, in the region of decrement; *c*, in the non-narcotized portion distal to the narcotizing chamber.

WITH ANELECTROTONUS			WITH CATELECTROTONUS		
Region near electrode	Region of decrement	Distal region	Region near electrode	Region of decrement	Distal region
Irritability decreased Conductivity decreased	Irritability decreased Conductivity decreased	Irritability decreased Conductivity decreased	Irritability increased Conductivity increased	Irritability decreased Conductivity decreased	Irritability increased Conductivity increased

SUMMARY

1. Whereas, in a non-narcotized portion of the nerve, a moderate polarizing current causes an increase of irritability near the cathode, in a narcotized portion of the same nerve this current causes a decrease in irritability. The effect of catelectrotonus on irritability therefore is reversed during narcosis.

2. A similar reversal of effect occurs in the case of conductivity. Catelectrotonus decreases conductivity in a partially narcotized region.

3. Anelectrotonus causes a decrease in irritability in a partially narcotized portion of the nerve, as in the non-narcotized nerve. Thus there is no reversal of effect as in the case of catelectrotonus.

4. Anelectrotonus similarly decreases the conductivity in a partially narcotized region, as in a non-narcotized region. This decrease

[1] The effects immediately after the make or break of the polarizing current are not shown here.

of conductivity is especially marked immediately after closing the polarizing current.

5. The time interval which elapses before anelectrotonic decrease in conductivity appears in a distant "partially" narcotized part of the nerve, to such an extent that the nerve impulse is put out, is longer than the time required for a nerve impulse to travel the same distance; thus an approximation of the speed of anelectrotonus (in the above sense) may be arrived at.

6. Concerning the interval which elapses before catelectrotonus causes sufficient decrease in conductivity in a distant narcotized portion to block an impulse, all that can be said is that it is longer than the time required for the impulse to travel the same distance.

7. A block for nerve impulses, set up by narcotizing a portion of the nerve, may be momentarily removed by the after-effect of anelectrotonus (effect produced at the anode immediately after turning off the polarizing current). Hence the after-effect of anelectrotonus in a narcotized portion of nerve is an increase of conductivity; this increase appears very quickly after the breaking of the polarizing current.

8. The after-effect of catelectrotonus in the narcotized portion is a decrease in conductivity.

9. Electrotonic changes are transmitted from a polarizing electrode through an intermediate narcotized region to the non-narcotized stretch beyond. This occurs when the narcosis is so deep that all impulses are blocked and even when the intermediate region of the nerve has been killed by narcosis. The electrotonic effects thus transmitted are of the same sign in the region beyond as they would be in the absence of a block, although in the intermediate region they may be changed in sign, as described above. If, instead of narcotizing, the intermediate portion of the nerve is cut and the ends are closely applied to each other, electrotonic changes in irritability do not spread to the neighboring piece. Prolonged exposure to concentrated zinc sulphate also blocks the spread of electrotonic effects.

For many suggestions and much valuable advice I wish to express my sincere thanks to Dr. H. J. Muller and to Dr. E. Altenburg.

EFFECT OF FATIGUE UPON THE HEART AND CARDIO-SKELETAL QUOTIENT

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On account of certain physiological properties of the heart muscle, it is generally stated that it can not be fatigued. The so-called refractory period is in large part responsible for the ineffectiveness of repeated stimuli to throw the heart into tetanus or fatigue. Skeletal muscles differ from heart muscle in that they very quickly show results attributable to fatigue, whether they are stimulated directly, or indirectly through their motor nerves. Also, one group of muscles may show the effects of fatigue, even though inactive, if other muscles are working and dumping their fatigue products into the circulation. These fatigue products are said to exert the same effects upon a muscle, whether they are produced in the muscle affected or in some distant muscle. That these products affect tissues other than muscle, e.g., nerve cells, is shown by the well known sensation of weariness that arises as a result of fatigue of muscles. The fact that a working group of muscles may, by its fatigue products carried in the circulation, produce manifestations of fatigue in other groups of muscles not working, suggested the question of the heart muscle also being affected by these fatigue products, and if so, would the effects be the same as those caused in other muscles? With this question in mind it was decided to investigate the effect of skeletal muscle fatigue upon the response of the heart muscle to quantitative electrical stimulation, utilizing a change in irritability of the heart muscle as an index of the effect of fatigue.

In a previous paper (1) was suggested the method of using a variation in a body constant as an index of the effects of physiological or pharmacological agents upon the heart or upon the skeletal muscles. The cardio-skeletal quotient was defined as a body constant which represents the relationship existing between the strength of stimulus necessary to arouse skeletal muscle (gastrocnemius) to contraction,

and the stimulus necessary to arouse heart muscle to contraction. In the work referred to above it was found that it takes approximately six times as strong a stimulus to make the frog's ventricle contract as it does to make its gastrocnemius contract. The figure found to express the cardio-skeletal quotient in those experiments is 0.17. In the control series of animals in the present investigation the cardio-skeletal quotient coincided exactly with the above figure.

Since the effects of fatigue on the heart were to be studied, it was desirable that in determining the cardio-skeletal quotient, one gastrocnemius be kept as nearly normal as possible. Following is the method used in this investigation. Frogs were used. First the brain was pithed, then the left leg was ligated close to the body. The skin below the ligature was slit and a circular incision made which cut all the tissues down to the bone. This was done in order to prevent any impulses passing under the ligature and thereby producing changes in the gastrocnemius muscle which was used as a control. The fatiguing process was next performed. This consisted in alternately stimulating the stump of the spinal cord and the muscles through the skin until the muscles failed to respond to stimulation through the cord. The cord was then pithed, and if extreme fatigue was desired, the muscles were stimulated through the skin until the responses were very feeble. In some instances where a more normal degree of fatigue was desired, the left leg was ligated first and after waiting a few minutes until pressure anesthesia of the leg had developed, the circular incision was made to the bone. The animal exhibited no signs of pain during this procedure. The frog was then induced to fatigue itself. This was done by placing the frog on its back, from which position it was rather difficult for it to recover because of the loss of function in one leg. It was found that this process could be carried on only for a short time, when the frog apparently would rather lie on his back and rest than turn over; so light electrical stimulation was then applied to the skin. This would arouse the frog to an effort of trying to escape. When this had been continued for some time and the frog showed signs of marked fatigue, the brain and cord were pithed. The heart was then exposed and prepared in the same manner as described in a previous paper (2). The threshold for the ventricle was determined immediately after introducing the electrodes and every five minutes thereafter for a period of twenty minutes; the threshold was then determined for the gastrocnemius immediately after introducing the electrodes and every five minutes thereafter for a period of twenty

minutes. In the experiments in the previous series it was found that the irritability of the ventricle and of the gastrocnemius become fairly constant after the lapse of fifteen minutes, and twenty minutes was the interval at which the cardio-skeletal quotient was estimated. In the present series several observations were made extending over a period of forty-five minutes to an hour.

Since in the present series one gastrocnemius was maintained in a normal condition (i.e., unfatigued), a few considerations should first be stated in regard to changes in the cardio-skeletal quotient under the conditions adopted. It is obvious that, the gastrocnemius remaining constant, any variation in the cardio-skeletal quotient must be the result of changes in irritability of the heart muscle. Hence a *decrease* in the cardio-skeletal quotient (i.e., from 0.17 to 0.12) must indicate a decrease in irritability of the heart muscle. The Z units are reciprocally related to the irritability of the muscle, thus, a *decrease* in irritability requires an *increase* in Z units necessary to arouse contraction. Conversely, it may be stated that if the gastrocnemius remains constant, an *increase* in the cardio-skeletal quotient indicates an increase in the irritability of the heart muscle or a *decrease* in the Z units necessary to arouse contraction. In other words, the plotted curve of the cardio-skeletal quotient will parallel the curve of irritability of the heart provided the gastrocnemius is kept constant. The Martin method of quantitative stimulation was used (3). Since the previous investigation showed that the results obtained by the Z units corresponded with the results obtained by the β units, the latter were not estimated in this series. A number of control experiments equal to the number of fatigue experiments were made.

In table 1 are shown the results obtained in the control animals. In the first two columns are shown the threshold, in Z units, of the ventricle immediately after introduction of the electrodes, also the corresponding cardio-skeletal quotient (C. S. Q.). In the succeeding columns are shown corresponding results at five-minute intervals. Table 2 shows the results of a similar procedure in fatigued animals. It will be noted that the average cardio-skeletal quotient in the control animals is the same (0.17) as was found in the series reported previously. This was estimated after the lapse of twenty minutes, as in the preceding series. The average cardio-skeletal quotient in fatigued animals is 0.22. Evidently the irritability of the heart is increased as a result of fatigue, if the cardio-skeletal quotient is estimated at the end of twenty minutes. The average threshold of the ventricles of

the fatigued animals, compared with the average threshold of the ventricles of the control frogs shows an increase of irritability amounting to 54.5 Z units or 24.7 per cent. Expressed in terms of the cardio-skeletal quotient, the irritability has increased from 0.17 to 0.22, a difference of 0.05 or 29.4 per cent. It may be that this is too high a per cent; thus it has already been shown (4) that the cardio-skeletal quotient is reliable in estimating the probable Z units of the heart, provided a series is used, thereby obtaining an average. Since the

TABLE 1

Showing the threshold stimulus of the ventricle in non-fatigued frogs immediately after introduction of the electrodes and at five-minute intervals thereafter for twenty minutes: the corresponding cardio-skeletal quotients (C. S. Q.) are also shown

EXPERIMENT NUMBER	0 MINUTES		5 MINUTES		10 MINUTES		15 MINUTES		20 MINUTES	
	Z.	C.S.Q.	Z.	C.S.Q.	Z.	C.S.Q.	Z.	C.S.Q.	Z.	C.S.Q.
5			161.0	0.27	212.5	0.26	238.0	0.26	255.9	0.25
9	87.5	0.13	156.6	0.08	171.5	0.07	188.0	0.07		
10			287.9	0.11	142.1	0.24	166.5	0.21	176.5	0.20
24	134.5	0.07	101.2	0.23	138.2	0.19	156.6	0.17	161.0	0.17
26	287.9	0.05	109.2	0.22	176.5	0.15	212.5	0.14	220.5	0.15
27	355.5	0.10	188.0	0.13	138.2	0.16	151.8	0.23	151.8	0.23
28	119.1	0.14	246.5	0.09	255.9	0.08	255.9	0.09	265.9	0.09
29	166.5	0.13	123.0	0.22	147.1	0.19	166.5	0.21	181.8	0.22
30	436.5	0.06	188.0	0.14	212.5	0.15	220.5	0.16	203.8	0.19
31	151.8	0.17	138.2	0.18	195.5	0.13	238.0	0.11	299.2	0.09
32	238.0	0.16	195.5	0.30	246.5	0 28	299.2	0.25	310.1	0.24
33	344.0	0.06	220.5	0.12	138.2	0.23	195.5	0.18	195.5	0.19
34	238.0	0.12	203.8	0.18	171.5	0.22	212.5	0.17	228.2	0.16
35	287.9	0.06	119.1	0.12	166.5	0.09	203.8	0.07	195.5	0.08
39	72.8	0.37	138.2	0.20	151.8	0.19	181.8	0.16	212.5	0.14
40	220.5	0.12	138.2	0.46	220.5	0.29	238.0	0.26	246.5	0.25
Average....	224.3	0.12	169.6	0.19	180.3	0.18	207.8	0.17	220.3	0.17

cardio-skeletal quotient, 0.17, in this series of control animals, also in the previous investigation, represented a normal condition, it might well be inferred that it represents the cardio-skeletal quotient in these animals before they were fatigued. On this basis the Z units of the ventricles in the fatigued frogs were estimated from the gastrocnemius Z units, using the cardio-skeletal quotient, 0.17. In these fatigued animals the gastrocnemius was kept normal, i.e., did not take part in the fatigue process. The results of the estimations show the average

Z unit for the ventricle (normally) would have been 196.0 instead of 220.0 as shown by the control frogs. As actually determined in the fatigued condition the threshold was 165.8 Z units. Thus instead of the rise in irritability amounting to 54.5 Z units, it amounts to 30.2 or 15.4 per cent. However, the change as expressed by the cardio-skeletal quotient percentage probably is not too high when it is realized that a change in the quotient of only about 5 per cent results in a corresponding change in ventricular Z units of over 15 per cent. In

TABLE 2

Showing the threshold stimulus of the ventricle in fatigued frogs immediately after introduction of the electrodes and at five-minute intervals thereafter for twenty minutes: the corresponding cardio-skeletal quotients (C. S. Q.) are also shown.

EXPERIMENT NUMBER	0 MINUTES		5 MINUTES		10 MINUTES		15 MINUTES		20 MINUTES	
	Z.	C.S.Q.	Z.	C.S.Q.	Z.	C.S.Q.	Z.	C.S.Q.	Z.	C.S.Q.
11	212.5	0.06	171.5	0.07	181.8	0.06	220.5	0.05	238.8	0.05
12	195.5	0.11	156.6	0.15	176.5	0.15	181.8	0.13	176.5	0.13
13	255.9	0.09	142.1	0.20	142.1	0.22	151.8	0.21	142.1	0.23
14	255.9	0.13	156.6	0.22	171.5	0.21	176.5	0.21	112.8	0.41
15	344.0	0.03	176.5	0.07	123.0	0.10	134.5	0.09	123.0	0.10
16	332.8	0.07	151.8	0.20	130.0	0.24	126.8	0.24	123.0	0.28
17	412.5	0.04	171.5	0.13	176.5	0.13	166.5	0.15	156.6	0.16
18	195.5	0.22	238.0	0.18	228.2	0.21	195.5	0.28	101.2	0.55
19	176.5	0.21	151.8	0.30	171.5	0.29	142.1	0.35	126.8	0.39
20	246.5	0.09	310.1	0.08	171.5	0.15	203.8	0.12	151.8	0.16
21	176.5	0.08	228.2	0.09	265.9	0.11	228.2	0.14	228.2	0.15
22	171.5	0.19	238.0	0.16	171.5	0.23	220.5	0.19	246.5	0.17
23	366.1	0.03	287.9	0.05	255.9	0.05	166.5	0.09	166.5	0.09
36	166.5	0.16	246.5	0.15	310.1	0.14	255.9	0.22	188.0	0.31
37	299.2	0.09	171.5	0.18	115.7	0.25	109.2	0.27	106.5	0.27
38	508.0	0.04	355.5	0.08	332.8	0.12	310.1	0.16	265.9	0.18
Average....	269.6	0.10	209.6	0.14	195.2	0.16	186.8	0.18	165.8	0.22

order to test further the validity of the cardio-skeletal quotient in making such estimations with a series of animals, the control animals in this investigation were used and the Z units of the ventricles were estimated from the gastrocnemius Z units, using the cardio-skeletal quotient, 0.17. The average of the Z units of the ventricles found in these estimations was 225.2. Looking then at the actual determinations the average was found to be 220.3, a difference of only 4.9 Z units or 2.2 per cent.

The increase in irritability of the heart as shown by the cardio-skeletal quotient estimated at twenty minutes after fatigue does not appear very great, but that it is definite is revealed by a more extended analysis of the cardio-skeletal quotient from the moment the electrodes were introduced. In the control animals the cardio-skeletal quotient at 0 and at five minute intervals thereafter was as follows: 0.12, 0.19, 0.18, 0.17, 0.17; in the fatigued animals it was: 0.10, 0.14, 0.16, 0.18, 0.22. In experiment 51 where the cardio-skeletal quotient was estimated for a longer period it was: 0.13, 0.12, 0.10, 0.18, 0.19, 0.23, 0.26, 0.28, 0.30, 0.34. Further evidence of this tendency of the cardio-skeletal quotient to rise in fatigued animals is shown by the average

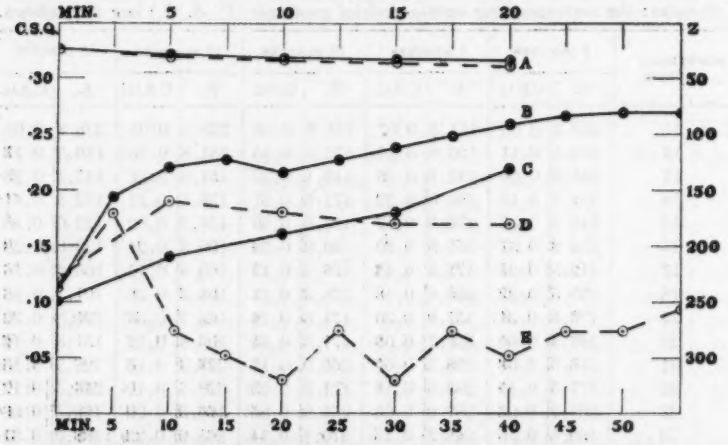

Fig. 1. Showing relative irritability of the heart in normal frogs (curves *D* and *E*), and in fatigued frogs (curves *B* and *C*).

obtained in a series of frogs which were moderately fatigued, or rather the fatiguing process was only carried to the extent which the animal could voluntarily produce. In this series the cardio-skeletal quotients were as follows: at twenty minutes, 0.20; at forty minutes, 0.36; at one hour, 0.48. This tendency is shown graphically in figure 1. In plotting the curves of irritability in those experiments where the observations were carried on for more than twenty minutes, the Z units were used in place of the cardio-skeletal quotient. Curve *C* shows the average cardio-skeletal quotient in the fatigued animals. The ordinates, designated at the left of the figure, represent the units of the cardio-skeletal quotient; the abscissas, designated at the top of the

figure, represent the time intervals. Curve *D* shows the cardio-skeletal quotient in the control animals. At *A* are plotted the curves of irritability of the gastrocnemii of the control (interrupted line) and the fatigued animals. The time intervals correspond to those of curves *C* and *D*. The ordinates, indicated at the right of the figure, represent the strength of stimulus in Z units. Curve *B* shows the irritability of the heart in experiment 46, a fatigued frog; curve *E* shows the irritability of the heart in experiment 48, a non-fatigued frog. The time intervals are designated at the bottom of the figure, the strength of stimulus in Z units is indicated at the right of the figure. These two curves, *B* and *E*, show practically the same result as does a composite curve of all the experiments but were chosen not only for that reason but because the initial stimulus of each happened to be the same, 238.0 Z units. Their curves may be taken as representative of the curve of irritability of the heart in moderately fatigued and in non-fatigued frogs. The irritability of the hearts in the fatigued animals, as shown in this series, is first low, then increases to normal and rises above normal. In animals where observations were carried on for a longer period than twenty minutes, the increase in irritability continued at least for an hour. In the control animals the irritability is at first low, rises above normal and then returns to normal where it remains for an hour or longer.

In order to see if this rise of irritability was simply due to excessive fatigue, a series of eight experiments was performed in which fatigue was produced that was not greater than the animal was capable of producing voluntarily. The cardio-skeletal quotients have been given above. Usually these experiments showed not so much depression of irritability at the beginning but rather an initial high irritability which became still greater as time went on. Experiment 46 shown in figure 1 at *B* is representative of this kind of an experiment.

All the data agree that the introduction of the electrodes into the heart muscle must produce at first a depression of irritability, since in both control and fatigued animals the curve of irritability rises during the first five minutes. In the normal animal it sinks back to a point which may be maintained for an hour or longer. In the fatigued animal it continues to rise. In other words, fatigue seems to cause the threshold of the heart to become more nearly the magnitude of that of a normal skeletal muscle. Thus in experiment 6, after an interval of twenty minutes it required only one and a half times as strong a stimulus to make the ventricle contract as it did to make the normal unfatigued gastrocnemius contract.

A further contrast was noted in the behavior of the normal hearts and those in the fatigued animals. In the former the heart would occasionally begin to beat spontaneously during the first five or ten minutes of the experiment, it would continue for a few minutes and then remain quiescent indefinitely. The fatigued hearts never showed this peculiarity during the first few minutes, but later when irritability, as shown by the strength of stimulus, had risen, it was not uncommon for it to start beating. The experiments in which spontaneous beating occurred were discarded.

The evidence obtained shows that the heart, like other tissues of the body, is affected by fatigue products. Unlike skeletal muscle, its irritability is increased rather than decreased. In the presence of large amounts of fatigue products the heart may also show a depression of irritability. This is indicated by the fact that the curve of the cardio-skeletal quotient in the extremely fatigued animals begins at a lower point than does that of the normal animals and does not reach the curve of the normal animals for about fifteen minutes. The curve indicates that when the amount of fatigue products is reduced to a certain point, then the influence is one of stimulation rather than depression. This is probably the condition in ordinary fatigue, as is shown at *B* in figure 1. Here the fatigue products could not have been so enormous as in curve *C* where the fatiguing was carried beyond the point where an animal could produce it voluntarily. In experiment 46 the animal did its own fatiguing, and it is noted that only its initial stimulus was as low as that in a normal heart; for a period of an hour it was considerably higher than the normal. This was true of all the experiments where the fatigue produced was of this moderate character. It is altogether probable that it is impossible for an animal to so fatigue itself that the resulting fatigue products would be present in sufficient quantities to *reduce* the irritability of the heart muscle. In moderate work it is conceivable that the increase in irritability of the heart has an adaptive purpose; thus with increased activity of the skeletal muscles, the heart muscle is influenced by the increase in fatigue products in the circulation and more readily responds to stimulation. Electrical measurements show this is true. Whether or not its response to chemical stimuli is also increased as a result of fatigue was not studied in this series. The continuation of the rapid beating of the heart following exercise may in part be explained by its increase of irritability due to the presence of fatigue products. It is also conceivable that continued fatigue as in overwork may give rise to car-

diac disturbances that are the result of increased irritability of the heart muscle.

The evidence obtained seems to justify the following conclusions:

1. The heart, like other tissues of the body, is affected by the products of fatigue.

2. Extreme fatigue, i.e., carried to a point impossible of attainment by voluntary efforts, causes a depression of the electrical irritability of the heart, followed by a rise of irritability which may continue for some time.

3. Moderate fatigue, i.e., carried to a point possible of attainment by voluntary efforts, causes a marked rise in the irritability of the heart muscle.

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THE OUTPUT OF EPINEPHRIN IN SHOCK[1]

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In most of the experiments the classical method of producing experimental shock, exposure with or without manipulation of the intestines, was adopted. "Manipulation" in our experiments included displacement, handling, pinching firmly between the fingers and thumb, flicking with the fingers, tapping with such objects as the handle of a scalpel, etc. Compression of the inferior vena cava till a condition of permanently low blood pressure was induced was also employed, since this method has been extensively used of late in the study of shock. We do not, however, consider this the most suitable method when the behavior of the adrenal is to be studied because of the possibility that a more or less prolonged period of passive congestion of the gland may be a disturbing factor. In one or two experiments a state of continued low blood pressure was induced by hemorrhage and in one experiment by the injection of "peptone." It is not to be imagined that exactly the same physiological changes are brought about when the blood pressure is permanently lowered in all these different ways. On the contrary, the condition produced by peptone injection obviously differs in important features from the condition which follows copious hemorrhage and this from the condition or conditions produced by the exposure of the intestines or partial occlusion of the cava, which are perhaps more properly termed experimental shock.

It was in order to increase the chance of finding a procedure which would grossly alter the rate of output of epinephrin that so many and

[1] About six months ago I was asked to become a member of the Sub-Committee on Shock (Medical Division of the National Research Council), the work more particularly assigned being an investigation of the output of epinephrin in that condition. The investigation has been made jointly by Doctor Rogoff and myself. A report summarizing the results presented in this paper was sent to the Committee September 28, 1918—G. N. S.

such different methods were employed. It is all the more notable that in none of the experiments was any evidence obtained that the rate of output was sensibly modified as compared with the initial rate. This result constitutes a new and striking illustration of the fact dwelt upon in previous papers (1) that the output of epinephrin is relatively stable and not easily influenced experimentally.[2]

The range of epinephrin output per kilogram per minute is, as determined by us, surprisingly narrow, not only in animals of the same species but in different groups. We have had the opportunity of determining the output in a baboon, weighing 2.51 kgm. It worked out at 0.0002 mgm. per kgm. per minute. The average found by us in cats, as assayed by intestine and uterus segments, is 0.00025 mgm. per kgm. per minute, and it is not very different in the dogs examined. Statements in the literature (6) to the effect that in shock conditions the output is much increased, even it may be to more than thirty-two times the initial output, are based upon faulty experimentation, the method of bio-assay adopted not being properly applied. The tracings accompanying the paper cited do not indicate in what manner the test objects (rabbit intestine "strips," meaning probably segments) were subjected to the action of the bloods. No quantitative estimations of the amount of epinephrin liberated per unit of time either before or after shock was induced are given. Nor is there any statement of the concentrations of epinephrin in the blood from which such estimations could be made. We are unable to follow the statement that shock can be induced without any lowering of blood pressure and "that in cases

[2] The fact that the output of epinephrin per minute remains practically the same with very different rates of blood flow through the gland is of itself sufficient to invalidate the contention of Popielski (2) against the existence of secretory nerves for the adrenals. He asserts that the result of stimulation of the splanchnics on the liberation of epinephrin is merely due to washing out already accumulated epinephrin through the increased blood flow. Since we have shown that increased output does not as a matter of fact accompany increased blood flow, this argument falls to the ground. It is astonishing that it should ever have been put forward. The complete abolition of the output of epinephrin by section of the nerves of the gland is certainly not accompanied by any marked diminution in the blood flow. We have not noticed that it is materially altered. And Burton-Opitz and Edwards (3) saw no definite increase in the adrenal blood flow during stimulation of the peripheral end of the splanchnic when a change of general arterial pressure was prevented. Popielski's statement that manipulation of the adrenal does not liberate epinephrin unless the gland is afterwards flushed out with blood, as by stimulating the splanchnics, is manifestly erroneous, as abundantly demonstrated in our own work (4), (5).

of shock accompanied by high blood pressure, the blood contains relatively a large amount of epinephrin." Some writers have asserted that in shock the store of epinephrin in the suprarenals is diminished. Apart from the fact that the methods used for the assay have ordinarily been inadequate, and that account has not been taken of the effect of anesthetics upon the store, there is no necessary relation between diminished store and increased output, as we have more than once pointed out (7).

A few of the protocols and some of the tracings used for the epinephrin assay in our experiments follow. The epinephrin was assayed by the method introduced by one of us (rabbit intestine segments) (8). In most cases the rabbit uterus segment was also employed to corroborate and sometimes to render more delicate the assay made on the intestine. As we have pointed out in previous papers, it is essential in order to obtain the best results from these methods of bio-assay, that numerous tests should be made with each blood. As a general rule it is advantageous to approximate to the epinephrin concentration in the blood specimen by determining a concentration of adrenalin which is definitely greater and one which is definitely less; then narrowing the interval between the higher and lower concentrations by a sufficient number of observations. It is then not difficult to obtain a concentration of adrenalin which gives a tracing approximately the same as that of the blood. This, however, is not always necessary in order to make a sufficiently close assay, if the higher and lower concentrations are not too far apart.

Condensed protocol

Dog 9. Male. Weight 6.6 kgm.
9.30 a.m. 50 mgm morphine hypodermically
10.45 a.m. Etherized. Put cannulae in trachea, carotid and jugular. Obtained specimen of indifferent blood from jugular
11.05 to 11.30 a.m. Made cava pocket,[1] tying abdominal aorta near the bifurcation

[1] In all of the experiments the cava pocket was made in a similar manner. The renal vessels and all veins entering the segment of cava between the liver and the point of insertion of the cannula were tied, leaving a pouch into which only the adrenal veins entered. The abdominal aorta was tied near the bifurcation. The cannula was inserted into the lower end of the cava and after pressing the blood out of the segment of cava the pocket was completed by a clamp, just beneath the liver immediately before collection of adrenal blood. After collecting each pair of specimens a fresh cannula was inserted into the pocket.

11.30 a.m. Arterial blood pressure 90 mm. of mercury. Now collected adrenal vein blood from the pocket
1st specimen 4.75 grams in 30 seconds (9.6 grams per minute)
2nd specimen 9.6 grams in 60 seconds (9.6 grams per minute)
11.35 a.m. Exposed intestines, with occasional manipulation
12.30 p.m. Blood pressure 68 mm. of mercury
12.32 p.m. Collected adrenal blood from pocket
3rd specimen, 2.35 grams in 30 seconds (4.7 grams per minute)
4th specimen, 7.3 grams in 90 seconds (4.7 grams per minute)
12.40 p.m. Replaced intestines in abdomen and fastened abdomen
12.45 p.m. Blood pressure 70 mm. of mercury
1.10 p.m. Blood pressure 80 mm. of mercury. Again exposed intestines
1.25 p.m. Blood pressure 60 mm. of mercury
1.45 p.m. Blood pressure 55 mm. of mercury
1.50 p.m. Collected adrenal blood from pocket
5th specimen, 2.3 grams in 60 seconds (2.3 grams per minute)
6th specimen, 6.8 grams in 180 seconds (2.3 grams per minute)
Combined weight of adrenals, 0.848 gram.

The epinephrin assay was made on the 2nd, 4th and 6th adrenal blood specimens. As in all this work, a specimen was collected immediately before each of these (1st, 3rd and 5th), so as to wash the cava segment completely free from any blood entering it during the manipulation connected with the clipping off of the pocket. The flow was not interrupted at all between the collection of the 1st and 2nd, 3rd and 4th or 5th and 6th specimens. After each pair of specimens had been obtained a fresh cannula was inserted into the cava so as to be ready for the next collection. In figures 1 to 6 are reproduced some of the tracings used for the assay. Those of the 6th specimen are given more completely than the others so as to illustrate once for all the manner in which the concentration of epinephrin in the blood is approximated to.

Figure 1 shows that the 2nd specimen, collected before the induction of shock, is notably weaker than the 4th, collected one hour after exposure of the intestines with a blood pressure of 68 mm., and that the 4th specimen is weaker than the 6th, collected two and one-quarter hours after exposure of the intestines, with a blood pressure of 55 mm. or less. This figure is reproduced to emphasize the fact that a qualitative comparison of tracings can never be used to determine whether a change in the rate of output of epinephrin has occurred, a point habitually neglected by some writers on this subject.

Although the much greater inhibition of the intestine segment produced by the 6th specimen than by the 2nd might suggest to a reader

unfamiliar with these methods of assay that the output had been much increased after shock, the assay proved that as a matter of fact there was no change. Figure 2 shows that the 6th specimen (observation 16) is much stronger than 1:3,750,000 adrenalin (observation 12), and stronger than 1:2,500,000 (observation 14). The tracings reproduced in figure 3 demonstrate that the 6th specimen (observation 48) is stronger than 1:1,875,000 adrenalin (observation 50). Figure 3-A shows that it

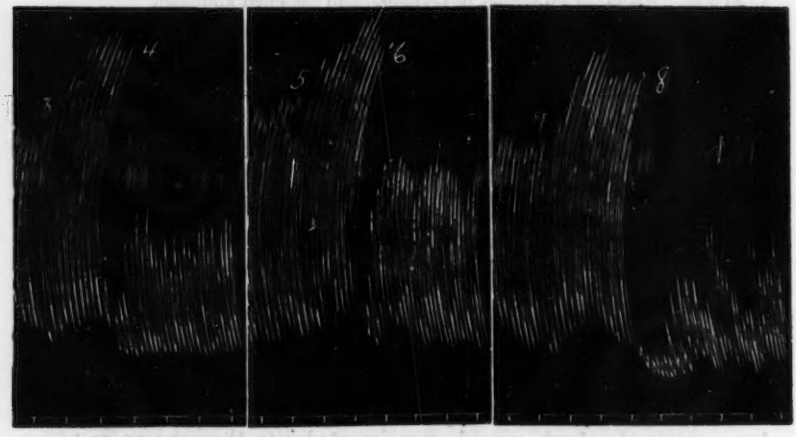

Fig. 1. Intestine tracings. Bloods from dog 9. At *3*, *5* and *7* Ringer was replaced by jugular blood and this was replaced at *4* by the 4th adrenal blood specimen (collected after 1 hour of exposure of intestines, blood pressure 68 mm. of mercury); at *6* by the 2nd adrenal blood specimen (collected before exposure of intestines, blood pressure 90 mm. of mercury) and at *8* by the 6th adrenal blood specimen (collected after 2 hours and 20 minutes exposure of intestines, blood pressure 55 mm. of mercury). All the bloods were diluted with 3 volumes of Ringer. As in all the tracings, time is marked in half-minutes. (Reduced to one-half.)

does not differ greatly from 1:1,250,000. The 2nd specimen is shown in figure 4 to be stronger than adrenalin 1:8,750,000. A pair of observations made immediately thereafter (not reproduced) proved that the 2nd specimen was stronger than adrenalin 1:7,500,000. A pair of observations made immediately before those in figure 4 indicated that the concentration of epinephrin in the 2nd specimen did not differ much from 1:6,250,000, and this was confirmed by later observations (not reproduced) on the same intestine segment.

Fig. 2. Intestine tracings. Bloods from dog 9. At *11*, *13* and *15* Ringer was replaced by jugular blood and this was replaced at *12* by jugular blood to which had been added adrenalin to make a concentration of 1: 3,750,000; at *14* by jugular blood with adrenalin added to make a concentration of 1: 2,500,000 and at *16* by the 6th adrenal blood specimen, which is stronger than 1: 2,500,000 (from other observations twice as strong). The difference between 14 and 16 appears relatively small simply because the inhibition at 14 is already so great. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

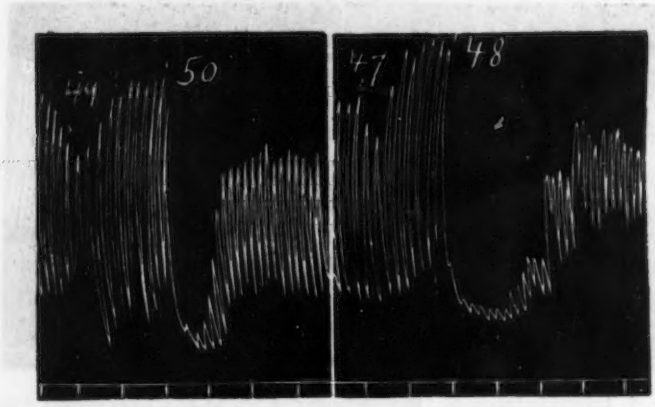

Fig. 3. Intestine tracings. Bloods from dog 9. At *47* and *49* Ringer was replaced by jugular blood and this was replaced at *48* by the 6th adrenal blood specimen and at *50* by jugular blood to which was added adrenalin to make a concentration of 1: 1,875,000. The bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

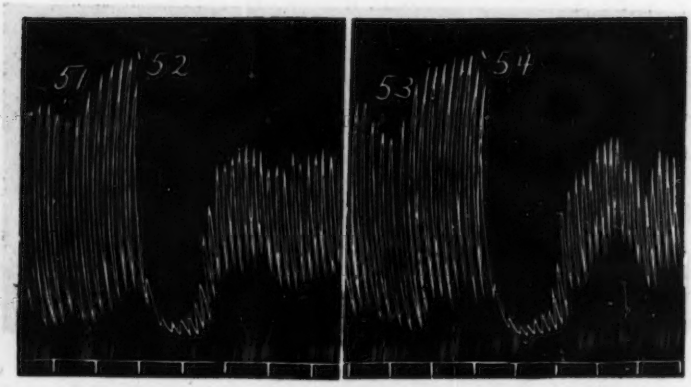

Fig. 3-A. Intestine tracings. Bloods from dog 9. At *51* and *53* Ringer was replaced by jugular blood and this was replaced at *52* by jugular blood with adrenalin added to make a concentration of 1: 1,250,000 and at *54* by the 6th adrenal blood specimen. The bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

Fig. 4. Intestine tracings. Bloods from dog 9. At *27* and *29* Ringer was replaced by jugular blood and this was replaced at *28* by jugular blood to which was added adrenalin to make a concentration of 1: 8,750,000 and at *30* by the 2nd adrenal blood specimen (collected before exposure of intestines). All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

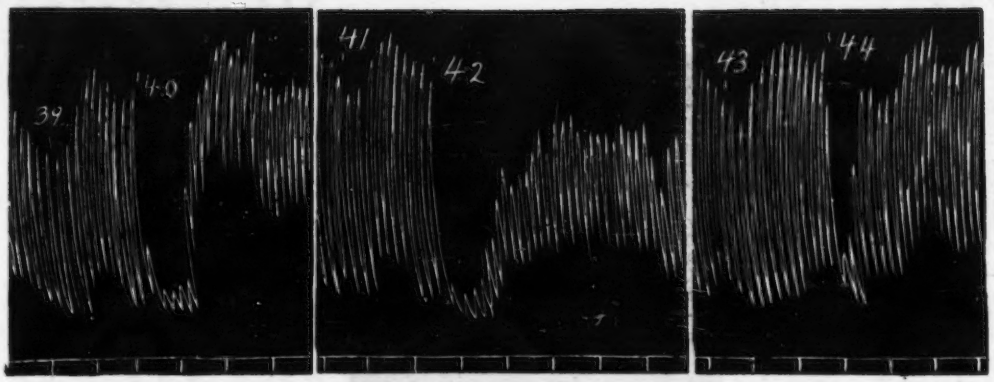

Fig. 5. Intestine tracings. Bloods from dog 9. At *39*, *41* and *43* Ringer was replaced by jugular blood and this was replaced at *40* by the 4th adrenal blood specimen (collected after 1 hour exposure of intestines, blood pressure 68 mm. of mercury); at *42* by jugular blood to which was added adrenalin to make a concentration of 1: 2,500,000 and at *44* by jugular blood with adrenalin added to make a concentration of 1: 3,750,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to four-fifths.)

In figure 5 it is demonstrated that the 4th adrenal specimen (observation 40) is decidedly weaker than 1:2,500,000 (observation 42) and somewhat stronger than 1:3,750,000 (observation 44). It was confirmed by two other pairs of observations (not reproduced), one just before and the other just after those recorded in figure 5, that the 4th specimen was not much different from 1:3,750,000, probably a little stronger.

In figure 6 are given samples of uterus tracings showing that the 2nd adrenal specimen produces a greater increase of tone than the indifferent (jugular) blood; the 4th specimen a greater increase than the 2nd and

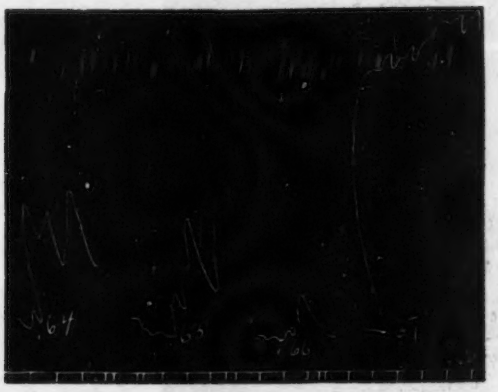

Fig. 6. Uterus tracings. Bloods from dog 9. At *64* Ringer was replaced by the 4th adrenal blood specimen; at *65* by the 2nd adrenal blood specimen; at *66* by jugular blood and at *67* by the 6th adrenal blood specimen. All the bloods were diluted with 2 volumes Ringer. (Reduced to one-half.)

the 6th a much greater increase than the 4th. Again it must be pointed out that the only conclusion which could be drawn from a comparison of the curves in figure 6, taken by themselves, would be that the concentration of epinephrin in the 6th specimen was greater than that in the 4th and the concentration in the 4th greater than that in the 2nd. It would be impossible to give any quantitative value to the differences. We know that in fact the 6th specimen was about three times as strong as the 4th and the 4th less than twice as strong as the 2nd. But this information is only obtainable by a systematic and rather laborious assay. Yet some writers from mere inspection of a few tracings revealing far less striking differences than these shown in figure 6, without any

attempt at quantitative estimation, have ventured to draw far reaching conclusions as to the influence of this or that factor in markedly increasing the rate of output of epinephrin. It may be safely assumed that when a paper does not give the amount of epinephrin liberated per unit of time, before and after the operation of the factor supposed to influence the rate of liberation but merely concludes that the rate has been greatly increased or greatly diminished, it does not contain any real evidence on the question.

Taking the concentration of epinephrin in the second specimen from dog 9 as 1:6,250,000 we get an output of 0.0015 mgm. per minute for the dog, or 0.00023 mgm. per kgm. of body weight per minute. Taking the 4th specimen at 1:3,500,000 we get 0.0013 mgm. per minute for the dog, or 0.0002 mgm. per kgm. per minute. Taking the concentration in the 6th specimen as 1:1,250,000 we get 0.0018 mgm. per minute for the dog, or 0.00027 mgm. per kgm. per minute.

Condensed protocol

Dog 6.	Weight 6.2 kgm.
11.00 a.m.	Morphine 25 mgm. hypodermically
1.00 p.m.	Etherized. Put cannulae in trachea, carotid and jugular. Made cava pocket
1.50 p.m.	Blood pressure 154 mm. of mercury
1.55 p.m.	Obtained indifferent blood from jugular. Now collected adrenal blood from the pocket
	1st specimen, 7.6 grams in 45 seconds (11.5 grams per minute)
	2nd specimen, 22.9 grams in 120 seconds (11.5 grams per minute)
	Blood pressure during collection of these specimens 123 mm. of mercury
2.00 p.m.	Exposed intestines; occasional manipulation
2.07 p.m.	Blood pressure 85 mm. of mercury
2.35 p.m.	Blood pressure 66 mm. of mercury
2.55 p.m.	Blood pressure 63 mm. of mercury. Collected adrenal blood from pocket
	3d specimen, 7.4 grams in 60 seconds (7.4 grams per minute)
	4th specimen, 16.0 grams in 150 seconds (6.4 grams per minute)
Combined weight of adrenals 0.905 gram.	

In this experiment the epinephrin assay showed that the 2nd adrenal blood specimen (taken before manipulation of the intestines was begun, with a blood pressure of 123 mm. of mercury) had a concentration of of 1:6,500,000. The output per minute for the dog was 0.0018 mgm., or 0.0003 mgm. per kgm. of body weight per minute. The 4th specimen collected nearly an hour after manipulation was begun, with blood

pressure of 63 mm. of mercury, had a concentration of 1:3,500,000, corresponding to an output per minute for the dog of 0.0018 mgm., or 0.0003 mgm. per kgm. per minute. A small sample of the tracings used in the assay is reproduced in figure 7, which shows that the 4th specimen (observation 17) had a concentration decidedly greater than 1:4,000,000 (observation 19), but somewhat less than 1:3,000,000 (observation 21). This was confirmed by other observations.

In a third experiment on a dog, weighing 7.25 kgm. (dog 7), a specimen of adrenal blood collected before exposure of the intestines, with a blood

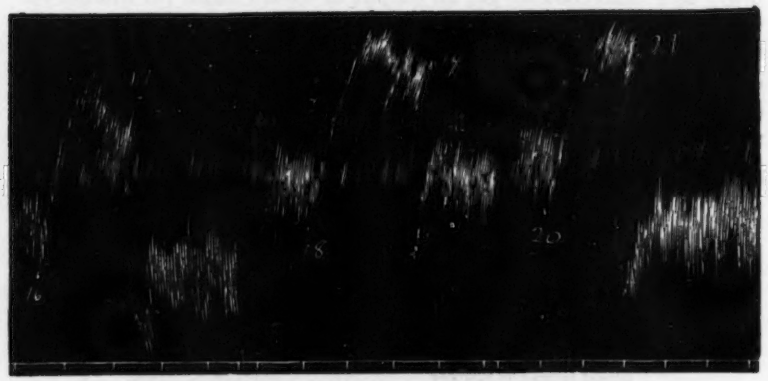

Fig. 7. Intestine tracings. Bloods from dog 6. At *16*, *18* and *20* Ringer was replaced by jugular blood and this was replaced at *17* by the 4th adrenal blood specimen (collected after 1 hr. exposure of intestines, blood pressure 63 mm. of mercury); at *19* by jugular blood to which was added adrenalin to make a concentration of 1:4,000,000 and at *21* by jugular blood with adrenalin added to make a concentration of 1:3,000,000. All the bloods were diluted with 2 volumes Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to four-sevenths.)

pressure of 113 mm. of mercury, had a concentration of epinephrin of 1:1,800,000, giving an output of 0.0018 mgm. per minute for the dog, or 0.00025 mgm. per kgm. per minute. A specimen, collected two hours after exposure of the intestines, was assayed at 1:1,250,000 epinephrin, corresponding to an output per minute for the dog of 0.0014 mgm., or 0.0002 mgm. per kgm. per minute. The blood pressure ten minutes before collection of this specimen was 72 mm. of mercury and at the end of collection it was 48 mm. of mercury. The flow during collection of the last specimen was probably too small, considering that

the concentration was approaching the possible maximum to allow the calculated output quite to equal the output before shock was induced.

Condensed protocol

Dog. 2. Male. Weight 7.3 kgm.
9.00 a.m. Morphine 50 mgm. hypodermically
10.50 a.m. Etherized. Put cannulae into trachea, carotid and jugular. Obtained indifferent blood from jugular
11.25 a.m. Started artificial respiration
11.45 a.m. Loose ligature placed around inferior cava, in thorax. Made cava pocket
12.40 p.m. Blood pressure 140 mm. of mercury.
12.50 p.m. Blood pressure 100 mm of mercury. Now collected adrenal blood from the pocket
1st specimen, 7.85 grams in 60 seconds (7.85 grams per minute)
2nd specimen, 16.1 gram in 120 seconds (8.0 grams per minute)
1.00 p.m. Partial occlusion of cava with occasional release, practiced at intervals for one and one-half hours. During this period the blood pressure gradually fell to 68 mm. of mercury at 2.30 p.m.
2.45 p.m. Collected adrenal blood from pocket
3rd specimen, 4.2 grams in 60 seconds (4.2 grams per minute)
4th specimen, 11.35 grams in 180 seconds (3.8 grams per minute)
5th specimen, 8.85 grams in 180 seconds (3.0 grams per minute)
Combined weight of adrenals 1.2 grams.

The second adrenal blood specimen (from dog 2) collected before occlusion of the cava, with blood pressure 105 mm. of mercury, was assayed at 1:6,500,000 corresponding to an output of 0.0012 mgm. per minute for the animal, or 0.00017 mgm. per kgm. per minute. The 4th specimen, collected after shock had been induced, contained 1:2,700,000 epinephrin, giving an output of 0.0014 mgm. per minute for the dog, or 0.00019 mgm. per kgm. per minute. The 5th specimen, collected with a blood pressure of 68 mm. of mercury, had a concentration of 1:2,100,000 corresponding to an output of 0.0014 mgm. per minute for the animal, or 0.00019 mgm. per kgm. per minute.

Condensed protocol

Dog 1. Female. Weight 11.3 kgm.
9.00 a.m. Morphine 100 mgm. hypodermically
10.45 a.m. Etherized. Put cannulae in trachea, carotid and jugular. Made cava pocket

11.30 a.m. Blood pressure 100 mm. of mercury. Now collected adrenal blood from pocket
1st specimen, preliminary specimen
2nd specimen, 17.4 grams in 30 seconds (34.8 grams per minute)
11.45 a.m. Bled from carotid (about 250 cc. blood withdrawn). Blood pressure just after hemorrhage, 60 mm. of mercury. Collected a preliminary specimen, then
3d specimen, 9.5 grams in 60 seconds
11.50 a.m. Closed abdomen with clamps
12.00 to 12.30 p.m. Pulse feeble and rapid; respirations very rapid (180 per minute)
1.50 p.m. Collected adrenal blood from pocket
4th specimen, 3.35 grams in 30 seconds (6.7 grams per minute)
5th specimen, 11.45 grams in 150 seconds (4.6 grams per minute)
Blood pressure just after collection of 5th specimen 40 to 50 mm. of mercury
Combined weight of adrenals 1.8 grams.

In this animal the 2nd adrenal blood specimen, obtained before hemorrhage, with the blood pressure at 100 mm. of mercury, was assayed at 1: 18,000,000 epinephrin. The rate of flow from the adrenals was large although no unligated small vein connected with the cava pocket was found *post mortem*. This accounts for the exceptionally low concentration of epinephrin. The output of epinephrin per minute for the animal for this specimen was 0.002 mgm. or 0.00018 mgm. per kgm. of body weight per minute. The 3rd specimen, collected immediately after hemorrhage, with the blood pressure at 60 mm. of mercury, had a concentration of 1: 3,300,000 of epinephrin, corresponding to an output of 0.0029 mgm. per minute for the dog, or 0.00025 mgm. per kgm. per minute. The 5th adrenal blood specimen, collected two hours after the hemorrhage, the blood pressure being 50 mm. of mercury at the beginning and 40 mm. of mercury at the end of collection, had a concentration of epinephrin of 1:1,500,000. This corresponds to an output of 0.003 mgm. per minute for the animal, or 0.00025 mgm. per kgm. per minute. Samples of the tracings used for the assays are reproduced in figures 8 to 12.

Figure 8 shows that at this stage the second adrenal specimen (observation 3) caused no definite inhibition of the intestine segment, which was not sensitive enough to detect the concentration of epinephrin contained in it, although later on it was assayed at 1: 18,000,000. The 3rd specimen caused a good inhibition (observation 5). It was assayed in other observations at 1:3,300,000. This is an excellent illustration of the danger of drawing quantitative conclusions from quali-

tative experiments. No doubt the inhibition in observation 5 was produced by a concentration of epinephrin five times as great as that which failed to cause inhibition in observation 3. But similar observations have sometimes been erroneously interpreted as indicating that a great "outburst" of epinephrin must have occurred when the specimen giving the positive reaction was being collected. In the present instance, as the assay showed, the rate of output of epinephrin was practically the same for both specimens. Figure 9 demonstrates that the 2nd adrenal specimen contained enough epinephrin to be detected and ap-

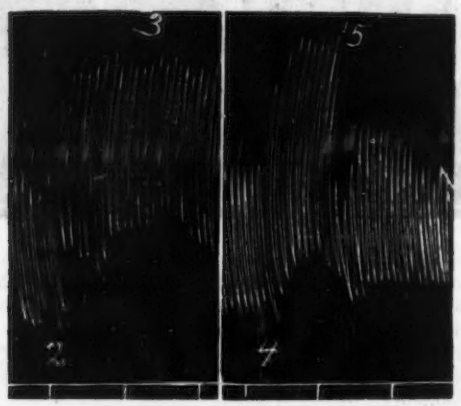

Fig. 8. Intestine tracings. Bloods of dog 1. At *2* and *4* Ringer was replaced by indifferent blood and this was replaced at *3* by the 2nd adrenal blood specimen (collected before hemorrhage, blood pressure 100 mm. of mercury) and at *5* by the 3rd adrenal blood specimen (collected a few minutes after hemorrhage, blood presure 60 mm. of mercury). The bloods were diluted with 3 volumes Ringer. (Reduced to two-thirds.)

proximately assayed by the same rabbit intestine segment when it had become more sensitive to epinephrin. The concentration is obviously less than 1:16,500,000 (observation 43) and greater than 1:20,000,000 (observation 45). These observations were confirmed by others and the concentration worked out at approximately 1:18,000,000.

In figure 10 it is shown that the concentration of the 3rd adrenal specimen is slightly greater than 1: 3,400,000. This was confirmed by other observations. It was taken finally at 1: 3,300,000. Figure 11 shows that the concentration of the 5th specimen is not very different from 1: 1,650,000 and figure 12, that it is considerably less than 1:1,250,000. It was finally taken as 1,500,000.

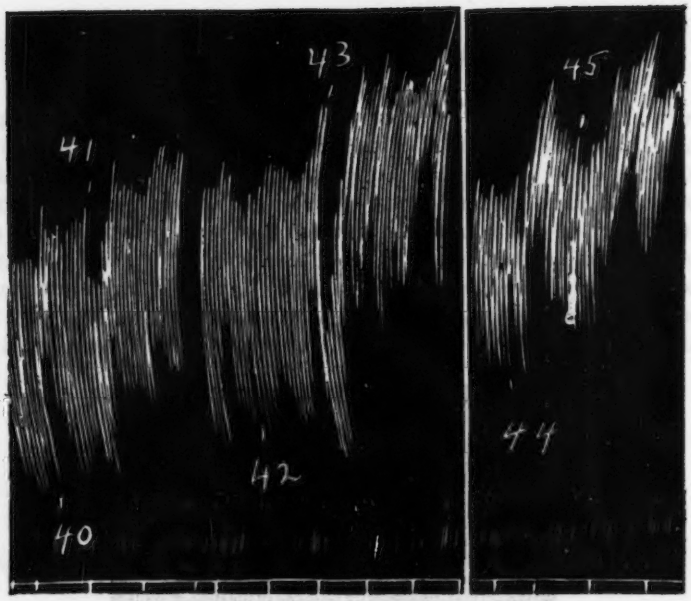

Fig. 9. Intestine tracings. Bloods from dog 1. At *40*, *42* and *44* Ringer was replaced by indifferent blood and this was replaced at *41* by the 2nd adrenal blood specimen (collected before hemorrhage); at *43* by indifferent blood to which was added adrenalin to make a concentration of 1: 16,500,000 and at *45* by indifferent blood, with adrenalin added to make a concentration of 1: 20,000,000 All of the bloods were diluted with an equal volume of Ringer (the adrenalin bloods after adding the adrenalin).

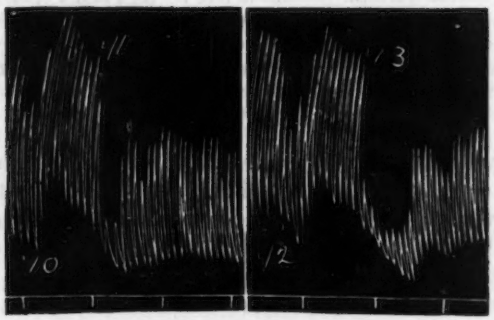

Fig. 10. Intestine tracings. Bloods from dog 1. At *10* and *12* Ringer was replaced by indifferent blood and this was replaced at *11* by indifferent blood to which was added adrenalin to make a concentration of 1: 3,400,000 and at *13* by the 3rd adrenal blood specimen (collected a few minutes after hemorrhage). All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to two-thirds.)

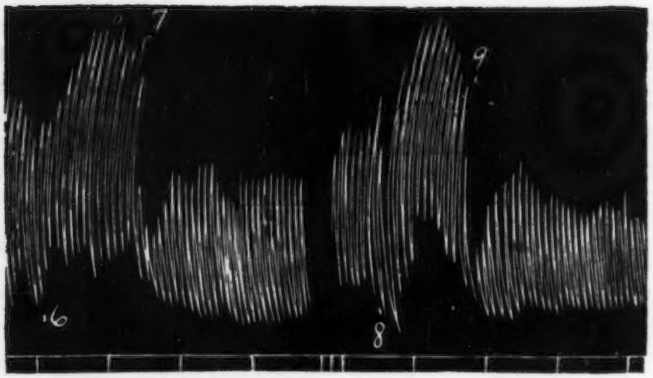

Fig. 11. Intestine tracings. Bloods from dog 1. At *6* and *8* Ringer was replaced by indifferent blood and this was replaced at *7* by the 5th adrenal blood specimen (collected 2 hours after hemorrhage, blood pressure 40 to 50 mm. of mercury) and at *9* by indifferent blood to which was added adrenalin to make a concentration of 1: 1,650,000. The bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to two-thirds.)

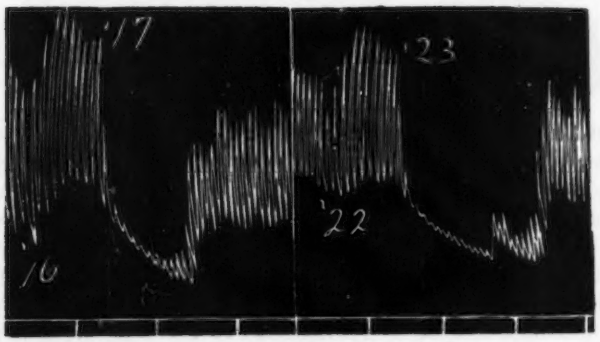

Fig. 12. Intestine tracings. Bloods from dog 1. At *16* and *22* Ringer was replaced by indifferent blood and this was replaced at *17* by the 5th adrenal blood specimen and at *23* by indifferent blood to which was added adrenalin to make a concentration of 1: 1,250,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

Condensed protocol

Cat 4. Male. Weight 3.255 kgm. Urethane anesthesia.

3.00 p.m. Put cannulae in trachea, carotid and femoral vein. Obtained indifferent blood from femoral vein. Started artificial respiration. Made cava pocket

3.40 p.m. Blood pressure before opening abdomen 120 mm. of mercury. Collected adrenal blood from the pocket
1st specimen, 4.85 grams in 20 seconds (14.5 grams per minute)
2nd specimen, 6.65 grams in 30 seconds (13.3 grams per minute)
3d specimen, 6.4 grams in 30 seconds (12.8 grams per minute)
Blood pressure at beginning of collection of 3d specimen, 104 mm. of mercury

3.45 p.m. Blood pressure after collection of 3d specimen, 70 mm. of mercury. Closed abdomen with clips

3.50 p.m. Blood pressure 105 mm. of mercury
Injected 1½ grams Witte's peptone (in 10 per cent solution) *via* external jugular vein
Blood pressure at end of peptone injection, 70 mm. of mercury

4.10 p.m. Collected adrenal blood from the pocket
4th specimen, 3.65 grams in 30 seconds (7.3 grams per minute)
5th specimen, 9.95 grams in 120 seconds (5.0 grams per minute)
6th specimen, 11.20 grams in 180 seconds (3.7 grams per minute)
Blood pressure after collection of 6th specimen, 60 mm. of mercury

4.17 p.m. Obtained indifferent blood from external jugular vein. (Label this "peptone indifferent blood")

4.40 p.m. Collected adrenal blood from pocket
7th specimen, 0.72 gram in 60 seconds
8th specimen, 0.68 gram in 60 seconds
Blood pressure during collection of 8th specimen, 28 mm. of mercury

Combined weight of adrenals 0.60 gram.

The 3rd, 5th and 8th adrenal blood specimens from cat 4 were carefully assayed, that is, one specimen collected before injection of peptone and two specimens after injection. For indifferent blood the "peptone blood" collected from the jugular was used in the assay of the 5th and 8th specimens. It was tested, however, against the indifferent blood obtained before injection of peptone and found not to differ materially from it as regards its action on the segments. The 3rd adrenal specimen assayed at 1:14,000,000 to 1:15,000,000 epinephrin, corresponding to an output of 0.0009 mgm. per minute for the animal, or 0.00027 mgm. per kgm. per minute. The blood pressure was 104 mm. of mercury at the beginning of collection of the 3rd specimen. The relatively low concentration is associated with a high rate of flow through the adrenals. No leak into the cava pocket from any other source could be detected *post mortem*. The 5th adrenal specimen, taken

twenty minutes after injection of the peptone, with blood pressure about 60 mm. of mercury, had a concentration of 1:6,500,000, giving an output of 0.0008 mgm. of epinephrin per minute for the cat, or 0.00025 mgm. per kgm. of body weight per minute. The 8th specimen was assayed at 1:850,000, giving an output of 0.0008 mgm. epinephrin per minute for the cat, or 0.00025 mgm. per kgm. per minute. Although the concentration in this specimen approached the possible maximum concentration, owing to the small blood flow through the adrenals,

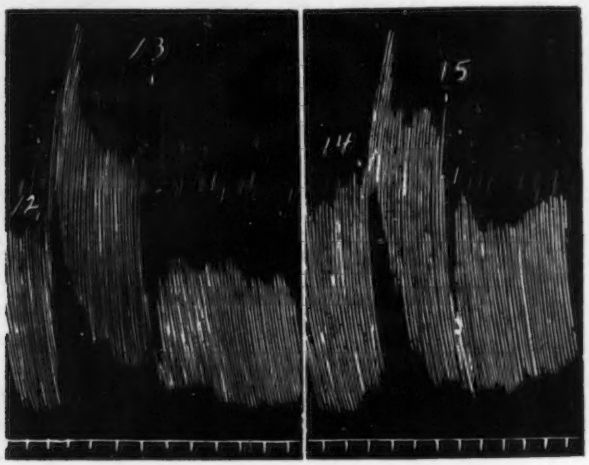

Fig. 13. Intestine tracings. Bloods from cat 4. At *12* and *14* Ringer was replaced by femoral vein blood and this was replaced at *13* by femoral vein blood to which was added adrenalin to make a concentration of 1: 14,000,000 and at *15* by the 3rd adrenal blood specimen (collected before injection of peptone, blood pressure 104 mm. of mercury). All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

associated with the low blood pressure at the time of collection (28 mm. of mercury), the curves obtained permitted a good assay. The results were confirmed on the uterus segments. Some of the intestine segment tracings are reproduced in figures 13 to 16. Figure 13 indicates that the concentration of the 3rd specimen is somewhat less than 1: 14,000,000. In other tracings it was shown that 1: 16,500,000 was markedly weaker than the 3rd specimen, while 1: 14,000,000 was only slightly stronger. In figure 14 it is demonstrated that the 5th speci-

men was weaker than 1: 5,700,000. Other tracings showed that it was stronger than 1:7,000,000. In figures 15 and 16 are reproduced some of the tracings used to assay the 8th specimen. Figure 15 shows that 1: 1,400,000 (observation 55) was much too weak for the specimen and that 1: 700,000 (observation 57) was too strong. From figure 16 it is clear that the specimen was stronger than 1:1,000,000.

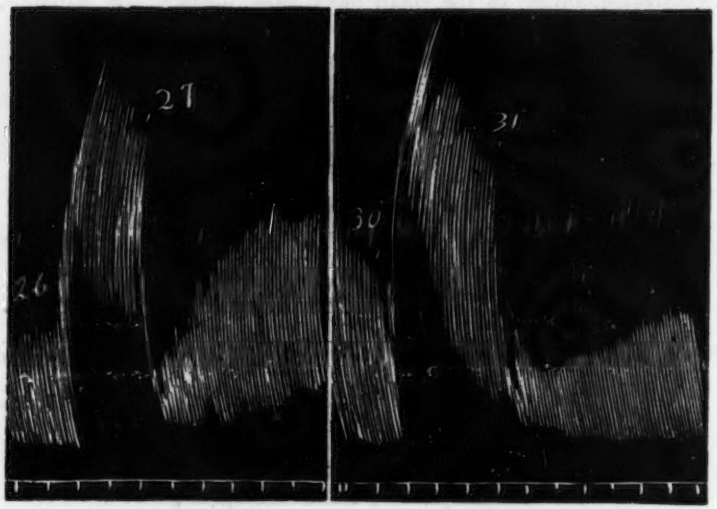

Fig. 14. Intestine tracings. Bloods from cat 4. At *26* and *30* Ringer was replaced by jugular blood and this was replaced at *27* by the 5th adrenal blood specimen (collected 20 minutes after injection of peptone) and at *31* by jugular blood to which was added adrenalin to make a concentration of 1: 5,700,000. All the bloods were diluted wih 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

The results of this investigation may be summed up in a sentence. In none of our experiments has evidence been obtained that any sensible change occurs in the rate of output of epinephrin from the adrenals in conditions of continued low blood pressure.

The only way in which we have hitherto been able to bring about an unequivocal increase in the epinephrin output (apart, of course, from that due to stimulation of the peripheral end of the splanchnic

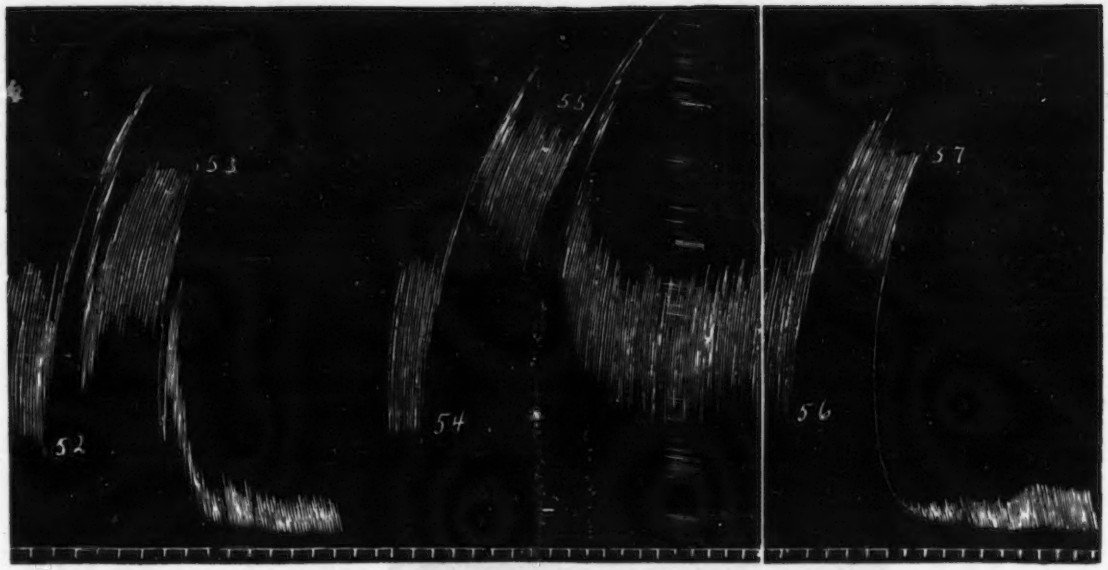

Fig. 15. Intestine tracings. Bloods from cat 4. At *52*, *54* and *56* Ringer was replaced by jugular blood and this was replaced at *53* by the 8th adrenal blood specimen (collected ¾ hour after peptone injection, blood pressure 28 mm. of mercury); at *55* by jugular blood to which was added adrenalin to make a concentration of 1:1,400,000 and at *57* by jugular blood with adrenalin added to make a concentration of 1:700,000. All the bloods were diluted with 6 volumes Ringer (the adrenalin bloods after adding the adrenalin.) (Reduced to two-thirds.)

nerve) is by the administration of strychnine or the intravenous injection of small quantities of concentrated solutions of salts (sodium carbonate), which cause a general excitation of the spinal cord. We purposely produced such an excitation in the hope of stimulating among other centers that which is associated with the secretion of epinephrin and is situated in the upper part of the thoracic cord (9). For this reason we have hitherto investigated the action of strychnine only in

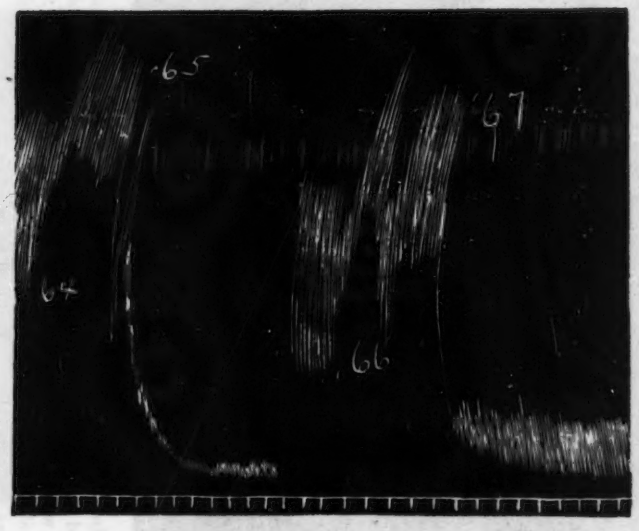

Fig. 16. Intestine tracings. Bloods from cat 4. At *64* and *66* Ringer was replaced by jugular blood and this was replaced at *65* by the 8th adrenal blood specimen and at *67* by jugular blood to which was added adrenalin to make a concentration of 1:1,000,000. All the bloods were diluted with 10 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

doses somewhat higher than the ordinary therapeutic doses, and have obtained a very marked increase of the rate of epinephrin output, both in the dog and cat, up to ten times the initial output. Asphyxia was excluded by artificial respiration. The effect of strychnine is not transient and may outlast for a considerable time the convulsant action, when such is present. The maximum increase is not necessarily found in specimens of adrenal blood taken immediately after the appearance of the symptoms of strychnine action on the cord. Thus, in one experi-

ment the output of epinephrin in blood collected immediately after the onset of the symptoms was shown to be three times the initial output, whereas one hour later it was four times the initial output. In another experiment the output was doubled with the onset of the strychnine symptoms but more than an hour later, when the symptoms had apparently completely subsided, the output was three times the initial output. In a third experiment, on a deeply anesthetized animal in which the dose of strychnine employed caused no convulsions, the epinephrin output was definitely increased in a blood specimen taken a few minutes after the injection of the strychnine, but the increase was twice as great in a specimen collected a little more than an hour thereafter. It is not always the case, however, that the later specimens show a greater increase than those collected soon after the administration of strychnine. In one experiment, for example, the output soon after the development of the strychnine symptoms was ten times the initial output, while a specimen taken three-quarters of an hour later showed six or seven times the initial output. Of course, the extent of the increase varies in different experiments. The subject is being investigated further, especially the action of smaller doses, and the details will be published in a subsequent paper. The possibility that the stimulating effect of strychnine upon the epinephrin output may enter as a factor into some of the pharmacological and therapeutic actions of the drug is obvious, but we have at present no evidence on this point.

SUMMARY

The rate of output of epinephrin in dogs and cats, after the blood pressure had been permanently lowered by exposure and manipulation of the intestines, by partial occlusion of the inferior vena cava, by hemorrhage and by "peptone" injection, was found to be the same as before the lowering of the blood pressure, within the limits of error of the methods used for assaying the epinephrin.

A marked increase in the rate of output of epinephrin was produced by strychnine.

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ON THE ELECTRICAL RESPONSES OF THE EYE TO STIMULATION BY LIGHT OF VARIOUS WAVE-LENGTHS

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HISTORICAL INTRODUCTION

Holmgren (1) is accredited with the discovery that when light is allowed to fall upon the eye of a frog which has been kept in the dark and again when the light is removed, there is an increase in the positive direction of the current present during darkness. He found that the strength of these retinal currents is, within limits, proportional to the intensity of the light; also, that the onset and removal of light is attended with the same electrical changes when the posterior half of the eyeball only is illuminated.

Dewar and M'Kendrick (2), who independently of Holmgren and at about the same time made investigations upon these electrical changes caused in retinae by light, proved experimentally, *a*, that the impact of light on the eyes of members of the following groups of animals, viz., mammalia, aves, reptilia, amphibia, pisces and crustacea, produces a variation amounting to from 3 to 10 per cent of the normal electromotive force existing between the corneal surface and the transverse section of the nerve; *b*, that this electrical alteration may be traced into the brain; *c*, that those rays which we regard as most luminous produce the largest variation; *d*, that the alteration of the electrical effect with varying luminous intensity seems to follow very closely ratios given by the psycho-physical law of Fechner; *e*, that the electrical alteration is due to the action of light on the retinal structure itself, as it is independent of the anterior portion of the eye, eliminating, therefore, the natural supposition that the contraction of the iris might produce such a result; and *f*, that it is possible by experiment to discover the physical expression of what is usually called in physiological language, fatigue.

Kühne and Steiner (3) investigated chiefly the reactions of the isolated retina. They found that the electrical change on lighting and darkening is a complex one. The reaction is divisible, they say, into two parts: the first one, due to the onset and continuance of illumination, consists of a negative variation preceded by a positive; and the second, caused by the disappearance of the light, consists of a simple positive variation. No current was obtained from the posterior half of the eyeball from which the retina had been removed, the pigment layer being left behind. Hence the pigment epithelium possesses no electromotive power.

Waller (4), whose brochure on *The Signs of Life* is most fascinating, concludes from his experimentation: *a*, A fresh normal eyeball manifests a positive current which gradually declines to zero and becomes reversed. By *positive current* Waller refers to the current of response due to the action of light on the retina which is from the nerve—that which is not directly stimulated by light—to the retina; that is, from the less excited to the more excited. *b*, On exposure to light the normal current, whether positive or negative, undergoes a positive variation. *c*, The magnitude of the response to light increases with the duration of illumination. *d*, The magnitude of the response to light increases with the strength of illumination. *e*, Colored lights act in the same direction and in accordance with their luminosity.

Gotch (5) made use of a capillary electrometer which recorded the rapid alterations of current present in the retinal responses. He divided the electrical reactions of the eye to light into three portions: *a*, the rise due to sudden illumination or the "on-effect;" *b*, the continuous change occurring during the continuance of the illumination, and *c*, a second rise due to the sudden change from light to darkness or the "off-effect." The results seem to indicate the localization of two photochemical substances in the posterior half of the eyeball, these being a substance reacting to light and a substance reacting to darkness. Each reaction is of the same type.

Einthoven and Jolly (6) used a string galvanometer and an arc as a source of illumination with suitable diaphragms. They conclude: *a*, The photo-electric reaction of an isolated eye is specially adapted to the study of the effect of stimuli of very different intensities. In their experiments light stimuli were used whose energy varied from 3.05×10^{-12} to 3.95×10^{-2} g. cal. per second. *b*, The form under which the photo-electric reaction manifests itself under different conditions gives ground for the supposition that there occur in the eye three sepa-

rate processes and that each of these may be dependent upon a separate substance. *c*, The first substance reacts more rapidly than the other two. On lighting it develops a negative, on darkening, a positive potential difference. *d*, The second substance reacts less rapidly than the first and in an opposite direction or sense. On lighting it develops a positive, on darkening, a negative potential difference. *e*, The third substance reacts in the same sense as the second but much more slowly. *f*, For each of the three substances the rule holds good that with moderate and strong light the energy of the stimulus increases much more rapidly than the energy of the reaction.

Other important researches, in a general way confirmatory of certain of the conclusions previously arrived at, have been made by Fuchs (7), DeHaas (8), Piper (9), Ishihara (10), Bruecke and Garten (11).

RÉSUMÉ OF EXPERIMENTATION AND OPINIONS UPON THE EFFECTS OF DIFFERENT WAVE-LENGTHS AND THE QUESTION OF COMPLEMENTARY OR ANTAGONISTIC INFLUENCES

Since we are particularly interested in this research in the investigation of the effects of various colors or wave-lengths of light upon the retinal response and in the question of corresponding or antagonistic influences, such as would be demanded by the Hering theory, for example, it is pertinent to present briefly the experimental evidence obtained up to date upon these points.

Gotch (12) exposed an excised frog's eyeball which had been kept in the dark to the red end of the spectrum for 1.6 seconds: the response commenced after a delay of 0.225 second and reached a maximum of 0.00045 volt at 0.8 second. After a period of five minutes' rest the result was checked. During a further period of five minutes' wait the position of the chamber carrying the eye was shifted to the green region of the spectrum, to which the eye was exposed for 2.7 seconds. The response began after a delay of 0.18 second and reached a maximum of 0.0004 volt at 0.6 second. And so on for the violet end, the delay in response being 0.22 second and the maximum response 0.0003 volt at 0.6 second. In conclusion Gotch says that the maximum response to red light was 0.0004 volt, green light 0.0005 volt and violet light 0.00024 volt. By means of a slit the "amount of light passing through the grating used could be always adjusted until it corresponded in photometric value with that of the candle." In concluding his remarks upon these investigations he says (13):

If it is permitted to associate these results with the well-known theories of colour vision, then it is evident that the Young-Helmholtz theory which assumes three primary colour reacting substances, red, green, violet, is in remarkable accordance with the data just put forward. In one respect only are the photo-electric responses not in accord with that theory, for although the responses appear to show that there is a substance capable of being stimulated by darkness there is no fourth reacting substance postulated in the theory. On the other hand, this is the only fact which is in any sense applicable to the rival theory advanced by Hering and even this is not in accordance with the assumption of Hering that the sensation of black, although caused by stimulation, is brought about by a change of opposite chemical type to that produced by white. The photo-electric responses, being all of the same fundamental type, are not in harmony with the assumption that there are three reacting substances, a red-green one, a blue-yellow one and a white-black one, and that the process is of opposite sign (association or dissociation) in each member, a process of one sign occurring with red, blue or white, whilst that of the opposite sign occurs with green, yellow or black.

In his first paper Gotch (14) concludes that the results appear to indicate the localization of two photo-chemical substances in the posterior half of the eyeball, these being a substance reacting to light and a substance reacting to darkness. Each reaction, he says, is a change of the same type, but for the change to occur the eye must be previously adapted, *i.e.*, the substances must undergo phases of metabolism under conditions opposite to those which evoke the reaction effects.

Waller (15) writes:

We may see whether or no complementary colours have opposite electrical effects, and whether the excessive action of a given colour favours or disfavours the action of its complementary. . . . . The effects of all sorts of colours were of the same sign. The joint effect of two complementary colours was practically the sum of their separate effects. And the prolonged excessive action of a given colour fatigued the retina to that colour just as much or as little as to the complementary colour. All colours, in fact, as regards the electrical response they elicit from the retina, give that response of the same sign and seem to act in the same direction, more or less powerfully according as they are more or less luminous.

Thus, *e.g.*, in a given series of trials, the responses had the following relative magnitudes:

Red	4
Yellow	16
(White)	33
Green	12
Blue	9

Furthermore, all the experimental facts obtained—viz., the transitional responses from positive to negative, the immediate conversion from positive to negative by compression, the initial period of hesitation—point to the conclusion that the retina, when exposed to light, is the seat of two contrary electromotive changes. And Waller says,

> It matters little whether you imagine two single processes or one double process behind the movements of the machinery. . . . . We are all acquainted with Hering's theory of colour-vision setting forth that contrary processes of dissimilation and assimilation are aroused by complementary colours. . . . And whether or no we happen to believe that, e.g., a red light is katabolic and a green light anabolic, we assuredly do believe in what has been termed by Bernard the axiom of general physiology—that katabolic analysis and anabolic synthesis are inconceivable apart from each other. I think that we should not hastily admit that our double electrical change is presenting us to another aspect of a familiar if somewhat nebulous colour-theory: I think we have no ground for assuming that our conclusions mean anything more than the old axiom behind a new face. A process necessitates the anti-process, and if the process is attended by an electrical change of given sign, we may expect that the anti-process will entail an electrical change of opposite sign. The chemical changes taking place in living matter are in general reversible changes.

PURPOSES OF THE PRESENT EXPERIMENTATION

We are presenting such experimental evidence as we have been able to obtain, after a number of repetitions, which bears upon:

1. The directions of the electromotive forces,—i.e., whether the current is from the nerve to the retina or vice versa,—under exposures to varying wave-lengths of light.
2. The question as to whether or not the excessive action of a given monochromatic radiation will promote the subsequent action of its complementary.
3. The general physiological problem of dissimilation and assimilation, or katabolism and anabolism.
4. The possible bearing of the experimental results obtained upon the theories of color vision.

We shall not attempt in this paper any theoretical discussions as to the possible physical, chemical or physiologic explanations of the effects observed, since we are of the opinion that too few facts are at hand to warrant the addition of another guess to the many that already exist.

APPARATUS

The schematic arrangement of the apparatus which was used is shown in figure 1.

Optical apparatus. The source of light, *S*, was a carbon arc, hand feed, supplied by an alternating current dynamo, *D*. The quartz lens system L_1 threw the light upon a slit and thence to a lens L_2 and a prism *P* carrying on one face a grating replica. A continuous spectrum was formed upon a cardboard screen marked into wave-lengths and represented by *R Y G B* in figure 1. This whole optical arrangement is conveniently assembled in the spectrum projector manufactured by the Bausch & Lomb Company. One of these devices was used through this work.

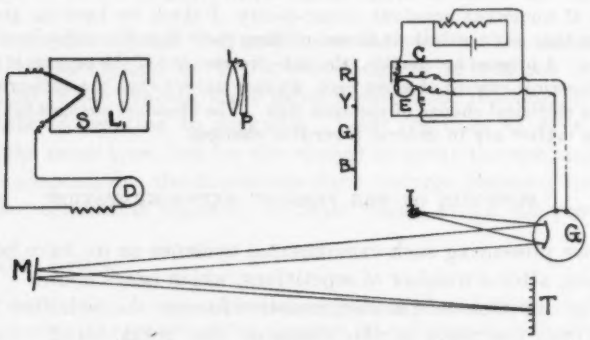

Fig. 1. Ensemble of experimental apparatus

The eyeball, enclosing case, etc. All eyes used were freshly excised from young dogs in healthy condition. In no case was experimentation with an eye started later than two hours after enucleation. The eyeball was allowed to rest on a hollowed-out pad of soft wax and was held in position by means of an arm swinging from a rod running the length of the enclosing case. To this same rod were fastened the nonpolarizable electrodes. Every precaution was taken to avoid mechanical pressure or injury to the eyeball.

The front of the containing case, *C*, was perforated by a small aperture carrying a swinging cover to admit or exclude light.

The electrodes made contact respectively with the cornea and with the nerve stump.

Galvanometer. The galvanometer was of the Thomson type and had a sensitiveness of 1.2×10^{-9} ampere, i.e., one millimeter deflection at one meter corresponded to a current of 1.2×10^{-9} ampere. Such a sensitiveness was not sufficiently high for the purposes desired judging by previous experimentation (15, p. 151). We therefore increased the sensitiveness about tenfold by using a concave mirror on the galvanometer and so placing a luminous source, *I*, as to cause a beam of light to be reflected from the galvanometer mirror across the room to a plane mirror, to be finally focussed on a transparent screen at *T* (fig. 1). This method enabled us to measure an indicated electromotive force of 0.00016 volt for one millimeter scale deflection.

Compensating potentiometer. When an excised eyeball is connected up through non-polarizable electrodes touching the cornea and nerve, a current of injury is found which must be compensated or eliminated. This can be accomplished as diagrammed in figure 2. A constant voltage cell may be short-circuited through a high resistance, *R*, from which any desired potential difference may be tapped off and applied across the eyeball in such a direction (by proper connection of the two terminal points of the battery to the eyeball) as to bring the galvanometer reading back to its initial zero point. In this manner, provided the compensating electromotive force demanded does not vary with time, the effects obtained by illumination are genuine and are not complicated by current or injury effects.

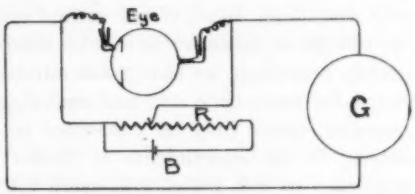

Fig. 2. Detailed diagram of compensating potential device.

Exposure to various parts of the spectrum. The case containing the eyeball was not disturbed during an evening's experimentation. The spectrum projection apparatus, which was fastened to a single supporting base, was turned so as to allow the eye to be exposed to any desired portion of the spectrum.

Electrical screening. The case holding the eye and the electrical portions of the spectrum projection apparatus were suitably screened with wire cages connected to earth to prevent spurious effects upon the galvanometer leads, etc. The leads, in turn, were wound non-inductively and reasonably well protected from stray electrical effects.

METHOD OF INVESTIGATION

a. Differences in present methods as compared with those previously pursued. In a general way, previous experimenters have exposed excised eyes (usually frogs' eyes) to luminous energy for periods of time varying from a few seconds to a minute and then to equal or longer periods of darkness and have thereby investigated the nature of the response and recovery curves. They have paid little or no attention to the general rise or fall of potential after stated periods of exposure. Hence we have, in general, exposed an excised eye to a certain portion of the spectrum for from four to five minutes and then have waited, the eye being screened, for an equal or longer period of time, before again exposing to a different portion of the spectrum. The general method of procedure has been to investigate the *end changes* in potential difference after alternate exposures to long (e.g., red) and short (e.g., blue) visual radiant energy. In our investigations on the effects of exposure to a color complementary to that used immediately preceding, we have made our experiments after the eye had been in use for some time and had ceased to show appreciable variations in response, hence may be conceived to have been fatigued to a given color. In the experiments of Waller we are led to believe that the responses to red, green, etc., were taken rather rapidly one after the other.

b. Sources of error. 1. In all such experimentation it is necessary to apply across the cornea and optic nerve trunk a compensating electromotive force to annul the current of injury. If is, of course, possible that this current decays with time or reverses its direction. To obviate this trouble we waited some little time before commencing our observations. Repeated series of observations with different eyes, the order of exposure to different radiations being considerably changed, aided in the eradication of false conclusions due to such an error.

2. The fact that the electrodes may be polarizable, whereas they should be absolutely non-polarizable. The usual form of clay boots with zinc and zinc sulphate were used. These are presumably the best known.

3. The effects due to mechanical pressure and jar. It has been found that mechanical pressure, etc., will modify or reverse the character of responses. Extreme care was exercised to prevent such pressures or jars.

4. Condition of the eye. The normal, fresh, uninjured eyeball gives entirely different types of response as compared with the partially injured or moribund eye.

5. The periodic variations of the sensitive galvanometer used. A type of galvanometer, such as the Thomson, when highly sensitive is subject to shifts of the zero in a rather rhythmic fashion. Naturally the repeated sets of observations, referred to above, aided in the elimination of this source of error. We refer to this source of error in particular, since some of the curves presented in this paper might be accounted for in their forms by this rhythmic variation of galvanometer swing were it not for the fact that several sets of observations showed that no such result was possible.

6. Fluctuations in the arc used.

All these sources of error would, however, be likely to be present or absent in greater or lesser degree in each different eye tested on different days and hence cannot vitiate the general results of the experiments to be described below.

RESULTS

a. The form of the photo-electric reactions to the initial light stimuli of different wave-lengths. A freshly excised dog eye was taken and, after preliminary arrangements, was initially exposed to a region of the spectrum such that λ5900 (sodium line) fell upon the center of the pupil. The total time of exposure was four minutes. The plotted results are shown in figure 3; in this figure the ordinates represent electromotive forces and the abscissas the time in seconds. The maximum value of the response to yellow light taken at any point on this curve, as judged with respect to its own minimum, is practically 20 divisions or + 0.0032 volt. The reason for the recurrent maxima, as *A*, *B*, *C*, etc., in figure 3, will be discussed later. The difference between the initial electromotive force (just before exposure, which was zero) and the final zero or reading after four minutes is + 10 divisions or + 0.0016 volt. The eye was then screened from the radiation but the process apparently continued such that in the end the final reading after six minutes of darkness was 30 divisions above the initial zero or +0.0048 volt. The lines drawn through the points *A*, *B*, *C*, etc., and the minimum points *D*, *E*, *H*, etc., are parallel and show the gradual *rise*, from the fiducial or arbitrary balance point, of the potential difference under exposure to the yellow (sodium light) region of the spectrum. This would indicate the constant growth of a positive elec-

tromotive force independent of the superposed maximum responses. It will also be noticed that in no instance is there a return to the initial zero, nor again any fluctuation indicating a reversal of sign in current or potential difference: this would be indicated on the plot by experimental values below the original zero point.

In contradistinction to these results obtained with λ5900, figure 4 shows the form of responses and potential differences when another freshly excised eye was initially exposed for four and a half minutes to the violet λ4310. The color represented by this wave-length is roughly complementary to that of λ5900, the effects of which have been described in conjunction with figure 3. The maximum value of the

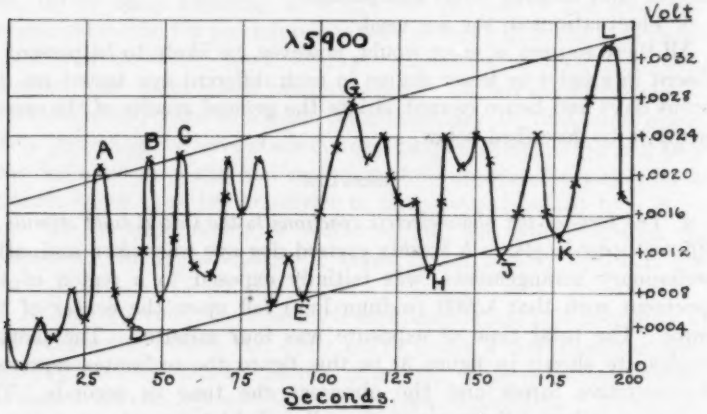

Fig. 3. Potential-time curve due to stimulation by λ5900

electrical response to λ4310 (at center of pupil) taken at any point during the first two or three minutes as judged with respect to its own minimum is practically −15 divisions or −0.0024 volt. The difference between the initial electromotive force—which was zero under the compensating potential device—and the final reading after four minutes' exposure was practically −15 divisions or −0.0024 volt. The eye was then screened from radiation and the end reading (after six minutes' rest) obtained, indicating a total difference between the initial and the end reading (over the whole period of time) of −12 divisions or −0.0019 volt. This is less than the general indicated difference, up to three minutes' exposure, of −0.0024 volt. However,

the concluding portion (from abscissa 180 to 270 seconds) shows a tendency for the contrary or opposite electrical effect (?) to exert itself more pronouncedly. The lines drawn through the points *A*, *B*, *C* and the maximum, electrically considered, points *D*, *E*, and *F* are parallel and show a gradual *fall* from the arbitrary balance starting-point under exposure to λ4300. This would indicate the growth of a *negative* electromotive force independent of the superinduced fluctuations giving rise to maxima and minima. It is also to be noted that there is, with one or two exceptions, no return to the initial zero nor any indications that there is a reversal of sign of potential difference or current except in the latter part of figure 4.

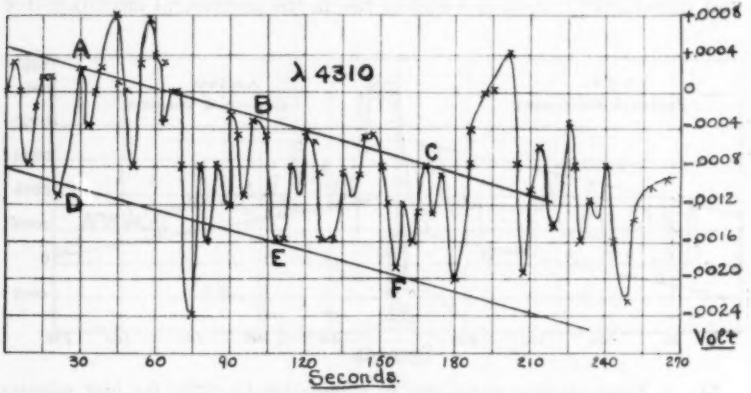

Fig. 4. Potential-time curve due to stimulation by λ4310

By comparison therefore we find that λ5890 produced *positive* potential differences and positive currents as well as a *gradual rise* in the zero, i.e., the positions of the minima in the various fluctuations observed, in a positive direction: λ4310, roughly the complement of λ5890, produced *negative* electromotive forces and a *gradual decline* (positively considered) or gradual increase (negatively considered) in the position of the arbitrary zero point in a negative direction. In the case of exposure to λ5890 the fluctuations of potential difference are always in such a direction that the maxima indicate *increased positive potentials at the nerve*, with returns to minima which are still always positively above the fiducial zero. In the case of exposure to λ4310, however, the fluctuations of potential difference are always in a direction indicative of *increased positive potentials at the retina*, hence *reversed* potentials as compared with the first named set of conditions.

b. The forms of the retinal responses produced by seriatim exposure to light stimuli of different wave-lengths. Figures 3 and 4 give initial effects in two different eyes. In figure 5 have been plotted seriatim the results obtained by exposure of the same eye to λ5900, first, for a period of four minutes, a period of six minutes' rest, and a second exposure to the blue-violet λ4860, the complementary color to λ5900, for a period of four minutes. The same general conclusions are to be drawn from the seriatim exposures as from the individual exposures in separate eyes. In the case of exposure to λ5890 there is a maximum rise of electromotive force in a positive direction of 0.003 volt: the difference between maxima and minima in the fluctuations of potential is 0.0024 volt (practically): there is a steady rise in the permanent electromotive

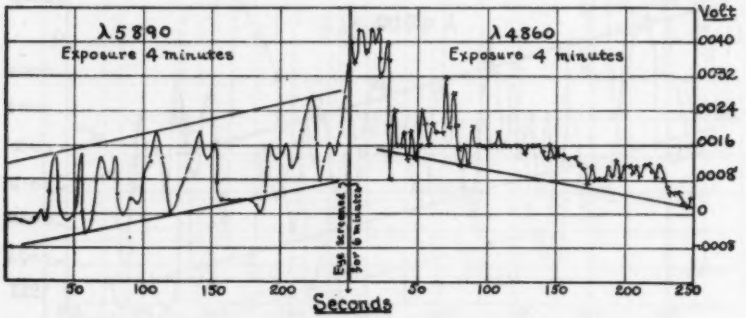

Fig. 5. Potential-time curve due to stimulation by λ5890 for four minutes followed by stimulation by λ4860 for four minutes.

force during the continuance of the illumination amounting to about 0.0012 volt. After the six minutes' rest, the initial exposure to λ4860 produced but slight, although decreasing, potential effects during the first 25 seconds, to be immediately followed by an increased electromotive force in an opposing direction such as to carry back the positive potential established under illumination by λ5890 toward the initial zero. The conflict of the two processes is very clearly indicated in the record of the responses to λ4860. The curve as a whole shows the establishment of a contrary electromotive force as compared with that established by λ5890; a decided reaction to λ4860 at the end of 25 seconds' exposure and the gradual decrease of the positive potential difference established under stimulation by λ5890. Furthermore,

both these curves, as well as others obtained by us (also see curve by Waller, *Signs of Life*, p. 26) show the first *pronounced* effect to light stimuli after 25 to 35 seconds of exposure.

Figures 6 and 7 present in graphical form the experimental results obtained when an eye was exposed to luminous stimuli of various wave-lengths seriatim. The manner of procedure in obtaining these curves was as follows: A fresh eye, an hour after excision, was connected into the galvanometer circuit and the galvanometer needle brought back

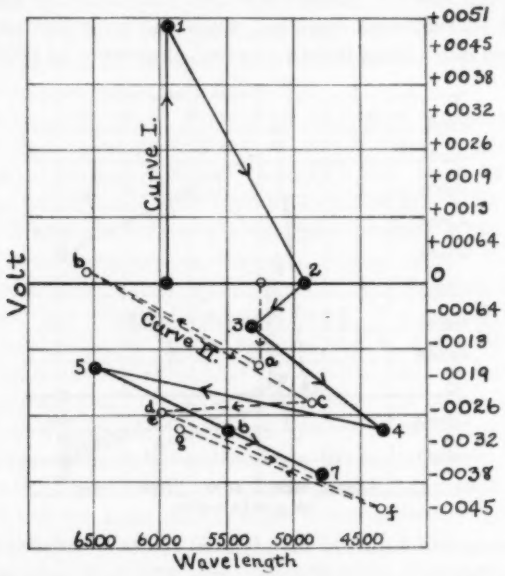

Fig. 6. Summarized results of the potential effects of seriatim exposures to various wave-lengths.

to its original zero through the agency of the compensating potential device. The eye was then exposed to a certain region of spectral energy for four minutes after which the eye was again stimulated by luminous energy of entirely different wave-length. This process was continued over several such steps in the case of each eye investigated. The initial stimulation was obtained from entirely different spectral regions in the four curves presented in figures 6 and 7. These curves are plotted with the abscissae indicating the region of light stimulation:

the ordinates represent the change in potential as registered by the galvanometer at the end of each cycle, namely the four minutes of exposure and the six minutes of rest. The order of stimulation, and regions used therefor, are indicated by the serial numbers *1*, *2*, etc., or by letters *a*, *b*, *c*. In curve *I*, figure 6, for example, the eye was stimulated by light of average (i.e., striking the center of the pupil) wave-length λ5890 (sodium yellow). The potential after four minutes' exposure and six minutes' rest had risen to 0.0049 volt as indicated at the point marked *1*. The eye was then stimulated by λ4860 for the same length of time and occluded, when the potential was found to have dropped down from 0.0048 to zero. Exposure to λ5300 reversed

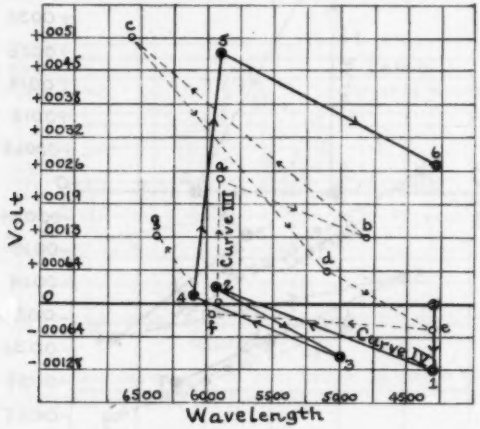

Fig. 7. Summarized results of the potential effects of seriatim exposures to various wave-lengths.

the potential and gave −0.007 volt; λ4350 carried the final reading to −0.0028 volt; stimulation by λ6500 reduced the potential to −0.0016 volt or carried it back toward the initial zero point; λ5500 increased the negative potential and λ4800 carried it to −0.0038 volt, at which point the experimentation was discontinued. In curve *II* of this figure, the initial stimulus was furnished by λ5250 (green) and ultimately gave a *negative* potential increase of −0.0016 volt indicated by the point *a*. Exposure to λ6563 (red) *reversed* the potential and gave a final value of 0.00032 volt; λ4860 *reversed* it and carried it back to −0.0024 volt and so on. The points marked *d* and *e* were dual

exposures to λ6000 and indicate that the second exposure to the same wave-length produced no further changes in potential. In fact the galvanometer needle remained practically quiescent during the whole of the second stimulation by λ6000.

It is of interest to note that in curve *I* λ4860 (point *2*) is practically the complement of λ5900 (point *1*) and in curve *II* λ5250 (point *a*), the complement of λ6563 (point *b*), and that in each of these cases the potential effects due to one are practically reversed and annulled by those due to the second member mentioned. The maximum potential effects were +0.0049 volt for λ5890 and −0.0016 volt for λ5250.

Curves *III* and *IV* of figure 7 are self-explanatory in view of the discussion in connection with figure 6.

We conclude, therefore, from these and other similar sets of curves obtained, that there is evidence of two processes operating in the production of retinal electrical potentials and that, in general, the shorter wave-lengths of light produce negative potentials (from retina to nerve) while the longer wave-lengths produce positive potentials (from nerve to retina).

c. Complementary colors and reactions. We have in two instances investigated the effects of luminous energy of a wave-length approximately complementary to the spectral region last used to stimulate the eye, under the condition that the eye had been under experimental use for some time and reached the stage when the responses to stimulation were of a minor character and did not show the pronounced and rapid changes of potential indicated in the initial stages of experimentation—such, for example, as diagrammed in figures 3 and 4. The history of the eye from which figure 8 was obtained is as follows: *a*, initial stimulation λ5900; *b*, λ4860; *c*, λ6563; *d*, λ5000; *e*, λ4300, involving a total time of 45 minutes. Immediately previous to the obtaining of this curve the eye had been exposed to λ4300 for four minutes. After a period of six minutes in darkness it was exposed to λ5890 (sodium light) for two and a half minutes. Upon "ironing out" the irregularities, the potential-time curve has the form of the heavy line *A-B-C-D-E*. At the point *E* (after 2.5 minutes) the eye was stimulated with λ5370 (green) and the general rise of the potential positively (i.e., from nerve to retina) is indicated by the portion of the curve *E F*, with a maximum electromotive force of +0.0020 volt. The results of this experimentation indicate quite clearly that λ5370 is more nearly the complement of λ4300 than is λ5890 and that λ5370

produced a decided change in potential and in the *reverse* direction as compared with the immediately preceding stimulant, λ4300.

Figure 9 gives a set of conditions somewhat the opposite of those shown in figure 8. After five exposures to various portions of spectral illumination in the manner detailed in connection with the discussion of figure 8, the eyeball was exposed for four minutes to λ5890. The further excitation under λ4300 produced but slight effect whereas, when the pupil was illuminated with λ4860, the increase of *negative* potential (i.e., from retina to nerve) is decisively indicated in the plotted results. Hence λ4860, the complementary color to λ5890, produced a decided change in potential and in the reverse direction as compared with the immediately preceding stimulant.

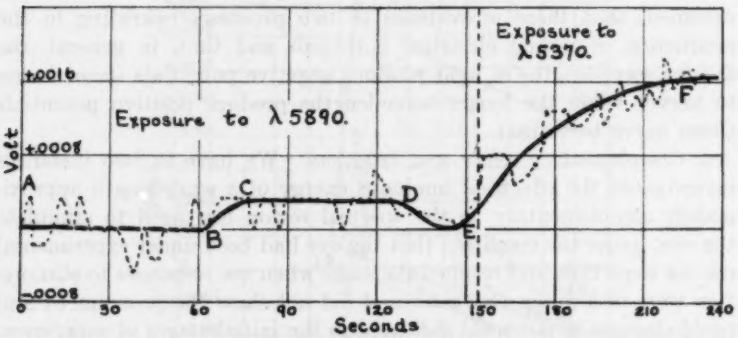

Fig. 8. Potential effects due to previous stimulation by λ4300 followed by stimulation with λ5890 and λ5370.

These facts are further substantiated in a general way by the results shown in figure 5, in which a stimulation by λ5890 is immediately followed by its complementary color, λ4860.

d. Possible explanations of the pronounced maxima and minima (or fluctuations) in the potential-time relations occurring during the early stages of luminous excitation. 1. Rhythmic variations of sensitive galvanometer system. Attention has been called to this under the heading "sources of error." Several sets of observations upon the "wandering of the zero" of the galvanometer needle were made on different days and evidenced much smaller values and entirely different periods as compared to the effects obtained with the eye under stimulation. Furthermore, a comparison of the results obtained in the initial stages

of experimentation, e.g., figure 3, with those obtained during experimentation with the same eye about an hour later, e.g., figure 8, show that galvanometer periodicity is not an appreciable factor and cannot account for the results obtained.

2. Mixed responses characteristic of transitional changes. Waller (15) has classified the responses to light as falling into three groups, the second of which is known as the *transitional* type; that is to say, transitional between the normal and the compressed or partially injured eye. This transitional type of response gives, according to Waller, potential-time responses which bear some similarity to those under discussion. It is possible, of course, that the experimental device used by us may have caused pressure, but extreme care was exercised to see that the eye rested easily on its support and the rods

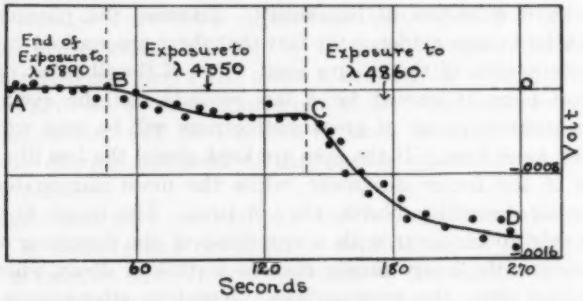

Fig. 9. Potential effects due to previous stimulation by λ5890 followed by its complementary, λ4860.

carrying the electrodes were so arranged as to permit of contact without any pressure. The eyes were always fresh, hence moribund conditions and changes can be cast out. Very suggestively Waller points out the composite effect of the superposition of two opposite and presumably synchronous electromotive forces. In nearly all the curves obtained by us we found that the first appreciable change in potential occurred after about thirty seconds' exposure and that during the first ten to twenty seconds there was some hesitancy in response. An inspection of Waller's curves, for example, shows that the maximum potential was attained in about half a minute. Previous experimenters do not appear, however, to have exposed the eye continuously for several minutes to stimulation but have preferred to alternately expose

and then rest the eye; hence a comparison of results in this particular is not wholly specious.

3. Fatigue. That which is to be presented in this paragraph may be but a process of reasoning from analogous visual effects, such as after-images, which may depend for their explanation upon the material presented under the succeeding subhead. If there are two separate and opposite sources of electromotive force, as seems reasonable from all the data obtainable, then continuous exposure may produce a cessation of or fatigue in one of the processes, thereby giving the opposite process the upper hand. Direct exposure of a living eye to illumination will, when carried out in such a manner as to prevent a wandering of the fixation, produce effects analogous to those fluctuations which we have obtained. By fixedly observing a brilliant light source, periods of cessation of luminous sensation will occur followed in a few seconds by a sensation of luminosity. Likewise the phenomena of positive after-images evidence the fact that there are marked variations in the colors, etc., of the images seen. For, if the filament of an incandescent lamp is viewed for a few seconds and the eyelids then closed, a positive image of great distinctness will be seen which will persist for some time. If the eyes are kept closed the less illuminated portions of the image disappear, while the more illuminated parts change color, becoming bluish, etc., in turn. The image finally disappears only to reappear with a repetition of the foregoing series of color changes: the image finally reaches a state of decay when it appears darker than the surroundings. Negative after-images, which possess the complementary colors to the original stimulus, may be thought of as fatigue or, again, as a metabolic change of opposite character to that produced by the original stimulus. The excised eyes used in our experimentation, being fixed and hence suffering retinal irritation and stimulation at one and the same region continuously, may reasonably be supposed to have undergone processes of fatigue and hence have exhibited "re-bounds" similar to positive after-image effects.

4. Polarization effects. The action of luminous stimulation upon the retina must lie without doubt in the ionization processes set up in the visual receptor cells. What these processes are or what the nature of the changes produced is still an enigma. However, ionization involves a dissociation into positively and negatively charged atoms and electrons. A stimulation by light must, therefore, set up a certain process of ionization whereby the positive ions may be thought of as

moving in general in one direction and the negative atoms (if such exist) and electrons as moving in opposite directions. Photo-electric activities of metals, such as sodium, show a discharge of negative electrons under the action of light, the rate of discharge depending upon the metal and the character of the illumination. However, we need not think of motion of both sets of ions but of one only, for example the electrons, and that membranes and tissues are more permeable to one than to the other of the ionized products. We may suppose that the retinal rods and cones contain certain electrolytes or ionizable substances which, in the absence of light, are ionized to a degree dependent upon the equilibrium constant of the reversible dissociation action. The introduction of light into the system alters the value of this constant by increasing the rate of the dissociating change without affecting that of re-association. It seems not improbable, therefore, that the passage of one set of charged particles out of the receptor cells along the nerve trunks will thereby increase the potential difference between retina and nerve and cause a building up of a surplus of the second kind or set of charged products of the ionization process which, by accumulation, will inhibit and in large measure sweep out the oppositely charged products of dissociation when the concentration becomes sufficient

We know that when one end of a wire is placed in contact with the cut end of a nerve and the other is touched to the ininjured nerve sheath, a current flows from the sheath to the cut portion. Hence the former is positive with respect to the latter. The Nernst-Lillie theory explains this by supposing the nerve tissues to contain an ionizable substance (or substances) the positive components of which diffuse through the sheath more readily than the negative. The sheath has, therefore, a differential permeability with respect to ions. When the cut end of the optic nerve is connected by a wire to the retina we find the retina to be more negative than the nerve end under certain conditions and *vice versa* under other conditions of stimulation. We must, therefore, it seems to us, postulate the existence of at least two ionizable substances in the receptor cells. One of these substances must give maximum ionization effects under yellow, for example, and the other similar effects under blue-violet stimulation. For one of the substances the negative ions of the receptor cells diffuse more readily than do the positive ones, while the reverse is true for the second substance.

Or, again, it is possible to look at the results from a different point of view, assuming only the opposing action of potentials set up at the receptor cells and the nerve fibers. L. T. Troland (16) in writing on the visual receptor process says:

> It is evident that if, in point of fact, the polarization of the receptor cells is opposite to that of the nerve fibres, there must be a constant tendency for these two tissue elements to depolarize each other, at their junction. In the case of the nerve, such depolarization will result in an impulse, which will pass along the fibre to the brain centers; in the case of the receptor cells, it will result in a momentary increase of permeability and the diffusion of a certain amount of substance through the cell membrane into the surrounding medium. After any single interaction of this sort, both the nerve fibre and the receptor cell will begin to recover their differential permeability as soon as the depolarization has reached its maximum. . . . . The reëstablished differential permeability and polarization of the nerve fibre consequently is maintained until the differential permeability of the receptor cells reaches, in the course of its recovery, a point at which the resulting receptor polarization is again able to neutralize that of the nerve fibre, and thus to cause a second depolarization of the latter. This intermittent action will obviously continue *ad infinitum*, if the electrolytes of the tissues are kept at the requisite concentrations.

POSSIBLE BEARING OF THIS EXPERIMENTATION UPON HERING'S THEORY OF COLOR PERCEPTION

The Hering theory assumes the existence in the retina of three visual substances, a whitish-black, a red-green and a yellow-blue substance. In each pair one color is complementary to the other. The theory also assumes that the different colors affect the different substances differently. Red stimulation induces a katabolic (dissimilirung) in the red-green substance, giving rise to a sensation of red, while green light gives rise to a constructive or anabolic change (assimilirung) in the same manner, producing the sensation of green. In the same way katabolic changes in the yellow-blue substance induced by yellow light give rise to the sensation of yellow, while anabolic changes are induced by the shorter visual wave-lengths of light. These theoretical considerations of anabolism and katabolism find their experimental counterparts in the positive and negative potential effects produced by various wave-lengths of light as outlined in the preceding paragraphs. These experiments point strongly to the conclusion that there are two distinct and opposing processes which may be designated as assimilation and dissimilation.

In figure 10 we have reproduced a diagram from Foster's *Text-Book of Physiology* to illustrate the Hering theory of color vision. The line *r g*, which encloses a space shaded vertically, is intended to represent the effects of rays of different wave-length on the red-green visual substance. In the red, orange and yellow, up to λ5800, the effect is katabolic: beyond λ5800 the effect is anabolic or one of assimilation. Similarly, the line *y b* represents the behavior of the yellow-blue substance and is shaded horizontally in figure 10.

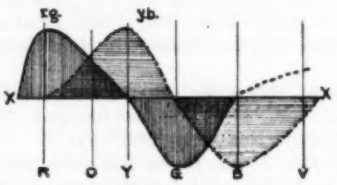

Fig. 10. Schema illustrative of Hering's color theory

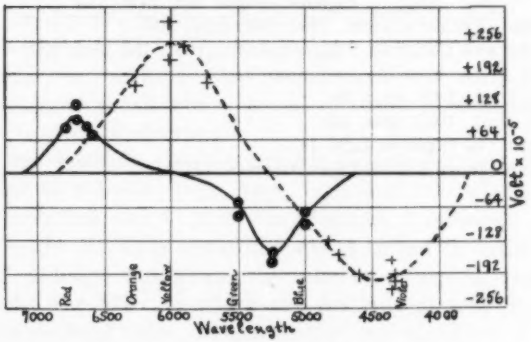

Fig. 11. End potential effects plotted against wave-lengths of light showing the similarity of potential curves to those demanded by the Hering theory.

Figure 11 gives a résumé of the retino-nerve potential differences due to various light stimuli obtained from various sets of observations made by us. A few data have been cast out in the plotting of these curves, otherwise the various values experimentally obtained have been plotted on a potential-wave-lengths sheet. The curves most nearly fitting the points have been drawn. Their similarity to the schema of the Hering theory is obvious.

In conclusion, we have been desirous of repeating portions of this investigation and of extending the researches to include experimentation upon living animals, thereby hoping to get more definite information upon the conditions of response in living eyes, but the exigencies of the times and the uncertainties surrounding research work have, up to the present, debarred us.

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CHANGES IN THE WEIGHTS OF THE VARIOUS PARTS, SYSTEMS AND ORGANS IN ALBINO RATS KEPT AT BIRTH WEIGHT BY UNDERFEEDING FOR VARIOUS PERIODS

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It has been shown that in young animals stunted by underfeeding certain of the systems and organs of the body show a remarkable tendency to continue their growth, others remain nearly unchanged, while still others lose weight to a variable extent (Aron, '11 and '14; Jackson, '15b; Stewart, '16 and '18a). The object of the present paper is to show the changes which occur in the weights of different organs and systems of the body in albino rats kept at birth weight (about 5 grams) by underfeeding for various periods, and also to compare these changes with those previously observed in larger rats during various periods and degrees of stunting.

Material and methods. In the present experiment 23 albino rats (Mus norvegicus albinus) were used, including 8 newborn controls (5 males, 3 females) and 15 test rats (8 males, 7 females). The sex and body weight for each individual rat is shown in table 1. The letters "St" indicate the series; the number preceding the decimal point indicates the litter to which the rat belongs and the number following the decimal point designates the individual rat.

The test rats were stunted from birth for periods ranging from 11 to 22 days of age (average 16 days) by subjecting them to repeated periods of fasting. This was accomplished by removing the test animals from the mother at successive periods as described in a previous paper (Stewart, '18a). Under normal conditions the body weight of the albino rat at 16 days (the average age of the test rats at the end of the experiment) would have at least trebled the birth weight. Thus the maintenance at birth weight for this period represents a condition of very severe inanition, which is maintained only with great difficulty. Many animals died during the course of the experiment and were not

utilized on account of the possibility of post mortem changes. The general plan adopted was to adjust the length of the alternate fasting and nursing periods in such manner as to keep the starved individuals

TABLE 1

Individual data for the albino rats used in the experiments

RAT NO.	USE AND SEX	BIRTH WEIGHT	WEIGHT AT AUTOPSY	AGE
		grams	*grams*	*days*
St 237.2	Control M	4.9	4.9	0
St 246.6	Control M	5.0	5.0	0
St 240.5	Control M	5.1	5.1	0
St 239.5	Control M	5.4	5.4	0
St 242.4	Control M	5.9	5.9	0
Average		5.3	5.3	
St 236.3	Test M	5.0	4.8	12
St 203.6	Test M	4.1	5.0	14
St 240.3	Test M	5.0	5.0	22
St 240.11	Test M	4.9	5.0	21
St 240.7	Test M	4.8	5.0	18
St 236.1	Test M	5.3	5.5	14
St 214.4	Test M	5.5	5.7	14
St 235.8	Test M	5.8	5.8	12
Average		5.0	5.2	16
St 238.1	Control F	5.0	5.0	0
St 240.1	Control F	5.1	5.1	0
St 239.7	Control F	5.5	5.5	0
Average		5.2	5.2	
St 215.5	Test F	5.0	4.0	11
St 235.1	Test F	4.8	4.8	12
St 235.4	Test F	5.0	5.0	12
St 239.10	Test F	5.4	5.0	20
St 214.6	Test F	5.5	5.0	13
St 237.3	Test F	4.1	5.1	18
St 214.2	Test F	4.6	5.1	18
Average		4.9	4.9	15

practically at birth weight. In this respect the experiments were very successful for, as is evident from tables 1 and 2, the average weights of the control and test animals are nearly equal.

TABLE 2

Showing the changes in average body length, tail ratio and weights of the various systems and organs in albino rats held at constant body weight by underfeeding from birth to 11 to 22 days of age

	MALES		FEMALES		SEXES COMBINED		AVERAGE PER CENT OF CHANGE
	Controls 5 newborn	8 Underfed 11-22 days	Controls 3 newborn	7 Underfed 11-22 days	Average of controls	Average of test rats	
Gross body weight, grams	5.26	5.22	5.20	4.86	5.24	5.05	−4
Net body weight, grams	5.08	5.03	4.98	4.64	5.04	4.85	−4
Body length, mm.	50.4	57.0	50.3	54.7	50.4	55.9	+11
Tail ratio, per cent.	31.3	40.5	32.4	43.6	31.7	41.9	+46
Head, grams	1.04	1.55	1.03	1.46	1.04	1.51	+45
Visceral group, grams	0.876	1.340	0.866	1.205	0.873	1.277	+46
Integument, grams	0.758	1.001	0.750	0.881	0.755	0.945	+25
Musculature and skeleton, grams	1.91	2.11	1.86	1.88	1.89	2.00	+6
"Remainder," grams	1.53	0.586	1.51	0.680	1.52	0.630	−59
Testes, grams	0.0027	0.0128			0.0027	0.0128	+374
Epididymis, grams	0.0016	0.0052			0.0016	0.0052	+225
Eyeballs, grams	0.024	0.057	0.023	0.060	0.024	0.059	+146
Brain, grams	0.217	0.525	0.231	0.474	0.223	0.501	+125
Kidneys, grams	0.0416	0.0874	0.0483	0.0794	0.0441	0.0837	+90
Spinal cord, grams	0.035	0.067	0.034	0.061	0.035	0.064	+83
Stomach and intestines (empty) grams	0.151	0.221	0.162	0.212	0.155	0.217	+40
Spleen, grams	0.012	0.019	0.011	0.012	0.012	0.016	+33
Hypophysis, grams	0.00056	0.00071	0.00047	0.00063	0.00052	0.00067	+29
Heart, grams	0.031	0.041	0.031	0.036	0.031	0.039	+26
Pineal body, grams	0.00028	0.00037	0.0003	0.00033	0.00029	0.00035	+21
Stomach and intestines (full) grams	0.334	0.413	0.383	0.427	0.352	0.420	+19
Thyroid, grams	0.00126	0.0015	0.0014	0.0013	0.0013	0.0014	+8
Ovaries, grams			0.00110	0.00116	0.00110	0.00116	+5
Lung, grams	0.088	0.097	0.083	0.079	0.086	0.089	+3
Suprarenals, grams	0.0021	0.0021	0.0017	0.0019	0.0019	0.0020	+5
Liver, grams	0.260	0.199	0.229	0.182	0.248	0.191	−23
Thymus, grams	0.0079	0.0042	0.0079	0.0038	0.0079	0.0040	−49

In comparing the data from the starved individuals with those from the controls, the slight difference in body weight (average about 4 per cent less in the test animals) has generally been disregarded, for the resultant error is small and does not modify the main conclusions based upon the data obtained.

The experiments were made in the Institute of Anatomy, University of Minnesota. The warm room in which the stunted rats were kept while fasting, and also the autopsy technique used are described in a previous paper (Stewart, '18a). The net "body weight" is the gross body weight less the weight of the contents of the alimentary canal.

For economy of space only average data are included in tables 2 and 3. However, copies of the original individual observations will be deposited at the Wistar Institute of Anatomy and Biology, Philadelphia, where they may be consulted if so desired.

Body length and tail length. Immediately after death the body (nose-anus) length and tail length of each rat were measured and recorded. Averages of the individual ratios $\frac{\text{(tail length)}}{\text{(body length)}}$ were computed from the data thus obtained and expressed as percentages (table 2). The ratio of tail length to body length is known as the "tail ratio."

According to the data in table 2, the body length of the rats kept at birth weight for 11 to 22 days (57.0 mm.) averages 11 per cent greater than the body length of the newborn controls (50.4 mm.). This indicates persistent skeletal growth in the young starved rats even though no increase in body weight was permitted. This result agrees with the previous observations by Jackson ('15b) and Stewart ('18a). Continued increase in body length during stunting by underfeeding has been observed not only in the rat, but also in calves (Waters, '08), and in dogs (Aron, '11). Also in malnourished children a strong persistent tendency to growth in body length (skeleton) has been noted by Variot ('07), Birk ('11), Aron ('14), Hess ('16) and others, even in cases where the body weight is stationary. This question is discussed more fully by Jackson and Stewart ('18).

The results (table 2) show the tail ratio to be considerably higher in the test than in the control rats. There is an apaprent increase in the tail length from a normal average of approximately 0.32 (31.7 per cent) of the body length in the newborn controls (sexes combined) to about 0.42 (41.9 per cent) in the starved individuals. This corresponds to an average increase of about 46 per cent in the absolute length of the tail,

as compared with an increase of 11 per cent in the body length. It is therefore evident that while the body continues to increase in length even during maintenance at birth weight, the elongation of the tail under these circumstances continues still more rapidly than that of the body, thus producing relatively long-tailed individuals.

Furthermore the data (table 2) show the tail to be relatively longer in the female than in the male rat, not only in the control but also in the test animals. Jackson ('15b) and Stewart ('18a) likewise noted the tail to become relatively long in young rats held at maintenance, and with a persistent tendency to average slightly longer in the female than in the male.

The relative elongation of the tail during underfeeding, although retarded in rate, apparently is an expression of the normal growth process, for according to Jackson ('15b) the tail ratio of the albino rat normally increases from an average of 0.36 (36 per cent of body length) at birth to 0.66 at 3 weeks, and is slightly greater in the female.

Head and body parts. The weight of the head (table 2) averages considerably higher in the test rats of each sex than in the younger controls of corresponding body weight. The combined data indicate an increase from an average of about 1.04 gram in the controls to approximately 1.51 gram in the test rats, an increase of about 45 per cent. As will be evident later, the increase (0.47 gram) can be attributed only partly to an increase in the weight of the brain and eyeballs. The extent to which the additional excess is shared by the integument, skeleton and musculature is uncertain from the data available, for no direct observations were made upon these parts for the head separately.

In older and larger rats underfed for various periods Jackson ('15b) and Stewart ('18a) noted a tendency for the head to increase in the majority of instances, even when the body weight was held constant. In general, however, the changes were not so marked as those observed in the present series of young individuals, in whom the intensity of growth is relatively much stronger. In a group of rats stunted by underfeeding from birth to 3 weeks, (body weight doubled), the average increase in the weight of the head was 16 per cent, as compared with normal controls of the same body weight (Stewart, '18a). This evidence of a relatively stronger growth tendency in the head region in the younger animals recalls the general law of cranio-caudal progression in growth and development, as formulated by Jackson ('09).

No direct observations were made with regard to the weight of the trunk and extremities, but with an increase in the weight of the head

during maintenance of constant body weight, the trunk and extremities collectively have necessarily decreased to a corresponding extent.

Visceral group, integument, musculature and skeleton, and "remainder." The weights of the visceral group (including abdominal and thoracic viscera, spinal cord, brain and eyeballs) and of the integument average considerably higher in the test than in the control rats of corresponding body weight, the average increase (sexes combined) amounting to 46 per cent for the viscera and 25 per cent for the integument. Also the musculature and skeleton together show a slight excess weight in the stunted animals in this case, however, amounting to only 6 per cent. The extent to which the change is shared by the skeleton and the musculature individually is uncertain for in this series the two were not weighed separately.

The "remainder" is obtained by deducting the weight of the integument, viscera, skeleton and musculature from the net body weight. It thus includes salivary and lymph glands, tongue, oesophagus, fat and body fluid but not the intestinal contents. A marked loss in weight of the "remainder" has occurred during underfeeding. The data indicate a decrease from an average of 1.52 gram in the controls to 0.63 gram in the test rats of corresponding body weight, a loss of about 59 per cent. The loss suffered by the "remainder" practically compensates for the combined increase observed for the above mentioned systems (correction being made for difference in average body weight).

A review of the literature dealing with the effect of inanition in general upon the parts and systems as well as the individual organs of the body in the rat and other animals is given by Jackson ('15a, '15b) and Stewart ('16, '18a).[1]

Organs gaining weight during fasting. From the data in table 2, it is evident that a considerable number of organs show a remarkable tendency toward continued growth in weight in young rats, even when an increase in body weight is prevented. The organs are arranged in order of their growth intensity when the body is held at birth weight in the first column of table 3. The most striking growth is noted in the case of the testes, epididymis, eyeballs and brain, the increase in the

[1] Statements in regard to the "remainder" after long periods of fasting (Stewart, '18a) should be changed to agree with the data, the latter being correct as published. Thus on page 328, line 8, "higher" should be changed to "lower." Also on page 331, line 6, change "six" and "ten" to "three" and "six" (weeks of age, etc.), respectively.

weight of these organs amounting to 374, 225, 146 and 125 per cent respectively. Of the other organs, the kidneys, spinal cord, empty stomach and intestines, spleen, hypophysis, heart and pineal body

TABLE 3

Changes in weight of organs in underfed albino rats compared with normal at same body weight

	MAINTENANCE FROM BIRTH TO 11-22 DAYS BODY WT. 5 GRAMS. (STEWART)	STUNTING FROM BIRTH TO 3 WKS. BODY WT. 10 GRAMS. (STEWART '18a)	STUNTING FROM BIRTH TO 10 WKS. BODY WT. 15 GRAMS. (STEWART '18a)	MAINTENANCE FROM 3 WKS. TO 10 WKS. BODY WT. 24 GRAMS. (JACKSON '15b)	STUNTING FROM 3 WKS. TO 400 DAYS BODY WT. 50 GRAMS. (STEWART '18a)
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Testes	+374	+188	+51	+34	−42
Epididymis	+225	+95	−6	−4	−32
Eyeballs	+146	+41	+66	+50	+73
Brain	+125	+60	+8	−0.5	+4
Kidneys	+90	+21	+38	+4	−6
Spinal cord	+83	+70	+70	+36	+40
Visc. group	+46	+28	+38	+0.9	+1
Head	+45	+16	+4	+6	+26
Stomach and intestines	+40	+17	+100	+28	−27
Spleen	+33	−49	+24	−42	−5
Hypophysis	+29	+33	+24	M +18 F +19	F +10 M −15
Heart	+26	−5	+27	−0.6	−10
Integument	+25	+0	−48	−36	−11
Pineal body	+21	−4	+12		−8
Thyroid gland	+8	+5	+4	−24	−36
Skeleton	+6	+19	+24	+28	+32
Musculature		+8	+10	+3	+4
Ovaries	+5	+83	+54	−27	+17
Suprarenals	+5	+60	+114	M +12 F +39	M +36 F +66
Lungs	+3	−26	−26	−15	+28 (?)
Liver	−23	+17	+64	+10	−7
Thymus	−49	−30	−80	−90	−90
"Remainder"	−59	−40	−23	−1.9	−33

Note. The data in the first column are from table 1.

also increase in weight considerably during maintenance of constant body weight from birth, the average percentages of increase being 90, 83, 40, 33, 29, 26 and 21 per cent respectively. The integument with

an increase of 25 per cent and the stomach and intestines including contents with an increase of 19 per cent also belong to this group.

The empty stomach and intestines show an increase from 0.155 gram in the controls to 0.217 gram in the test rats, a gain of about 40 per cent. When the contents are included with the stomach and intestines there is an apparent increase in the starved rats from a normal average of 0.352 gram to 0.420 gram, the increase amounting to about 19 per cent. The smaller gain recorded for the full than for the empty stomach and intestines is evidently due to the fact that the weight of the intestinal contents remained practically unchanged during the underfeeding, increasing from a normal average of 0.197 gram to 0.203 gram in the test rats, a gain of only about 3 per cent.

In the case of the pineal gland the apparent increase of 21 per cent in the test rats may be somewhat questionable, especially when the extremely small size of the organ is taken into consideration. Very small errors in technique of removal and weighing the organ may be responsible for the apparent increase in its weight.

On the whole, however, it is evident that during the severe inanition required to hold the body at birth weight the above mentioned organs continue to increase in weight at the expense of other parts of the body. In some instances the increase accomplished during starvation is very remarkable.

Organs remaining nearly constant in weight during fasting. In a smaller group of organs (see table 2) there is practically no growth in the rats kept at birth weight. The percentage increases (table 3, first column) are as follows: thyroid gland, 8; ovaries, 5; suprarenals, 5; and lungs, 3. The skeleton and musculature, which together show an increase of 6 per cent, also belong in this group. The average weights for these organs differ from those for the control animals by less than 10 per cent although in each case the average small difference indicates a slight apparent increase in weight of the corresponding organ.

Organs losing weight during fasting. Of the organs weighed individually, only two, the liver and thymus (tables 2 and 3) were observed to lose in weight in the rats kept at birth weight. The average data (sexes combined) indicate a loss of 23 per cent for the liver and 49 per cent for the thymus. As previously noted, the greatest loss is found in the "remainder" (decrease of 59 per cent) which, together with that of the liver and thymus, must counterbalance the gain in the other parts of the body since the body weight is held approximately constant.

DISCUSSION AND CONCLUSIONS

On comparing the results of the present experiments with those previously obtained in similar experiments upon older and larger animals a more complete survey than was hitherto available is obtained of the weight changes which occur among the various organs and parts of the body at various ages during inanition. This comparison brings out very strikingly the changing reaction undergone by the various individual organs at different ages when the individual is stunted in growth by underfeeding. The extent and character of this change for individual organs and systems is so well shown in table 3 that a detailed discussion of the individual organs is unnecessary. Special attention is called to certain points, however.

The visceral group as a whole averages 46 per cent increase in weight in the rats held at birth weight, as shown in column 1 of table 3. At later ages, and with longer periods of underfeeding (as shown in the successive columns of table 3) the tendency of the visceral group to increase persists, but becomes weaker. In the rats underfed beginning at the age of 3 weeks (last two columns of table 2), the visceral group barely holds its own in weight. In comparison with this general trend in the visceral group, the individual organs show great variability.

The testes show a remarkable increase of 374 per cent in rats kept at birth weight for various periods. In later periods of underfeeding the tendency to increase in the weight of the testes is progressively weaker, while in rats weighing approximately 50 grams at 400 days a loss of 42 per cent is recorded. Other organs, like the epididymis, brain, kidneys and alimentary canal, during the earlier periods of underfeeding exhibit a marked growth capacity, but later (like the visceral group as a whole) are barely able to maintain themselves, or in some cases actually suffer a loss in weight, under the conditions of the experiment. The eyeballs and spinal cord, however, not only increase markedly in weight in the younger underfed groups, but also tend to maintain strong growth tendencies with considerable persistency, even in the older groups. The thymus at all periods shows a marked loss in weight during underfeeding, while the other organs are variable.

The above mentioned increases in the weights of various organs and parts of the body during underfeeding have been observed to be accompanied also by continued structural development of apparently normal character. Thus in the case of the skeleton (Jackson, '15b and Stewart, '18a) continued growth during stunting has been shown

to be accompanied by continued development of apparently normal character (increasing proportion of solids, formation and fusion of epiphyses, development of third molar teeth). A progressive differentiation of the integument and external ear and opening of the eyelids before reaching the body weight at which this normally occurs has also been noted in young rats stunted by underfeeding (Stewart, '18a). The growth of the brain under similar conditions is likewise accompanied by normal differentiation of the parts as shown by the relative weight of the various segments (Stewart, '18b).

While the growth tendencies of the skeleton and musculature are apparently weaker in the present series held at birth weight than in subsequent periods, the integument and the visceral group show decidedly stronger growth capacities than at later periods of underfeeding (see table 3). A relatively enormous growth capacity is exhibited by certain organs during this earliest period. In older and larger rats considerable growth has previously been observed in the case of certain organs during underfeeding, but such remarkable changes as found in the present series (rats kept at birth weight) with increase of 374, 225 and 146 per cent (testes, epididymis and eyeballs respectively) have not hitherto been observed. As a general rule it appears that in most cases the growth tendency of organs during underfeeding is strongest during the youngest stages.

When individual organs are considered, however, it will be noted that there are some exceptions to this rule. For example, the liver shows a loss of 23 per cent in rats kept at birth weight (11 to 22 days) whereas in underfed animals weighing 10 and 15 grams at 3 and 10 weeks of age there is an increase of 17 and 64 per cent respectively. Also in the case of the ovaries and suprarenals there is apparently a lower growth tendency noted in the youngest group as compared with the marked increase found at 3 and 10 weeks of age in rats weighing 10 and 15 grams, respectively. Other organs, such as the spleen and lungs, are somewhat irregular.

In comparing the results of the present experiment with earlier investigations on the effects of underfeeding at later periods, the following generalizations seem justified. The "remainder" suffers a very heavy loss in rats held at birth weight by underfeeding, while the skeleton and musculature show a very feeble gain as compared with the tendency at somewhat later periods. The tendency of continued growth in the integument and visceral group during inanition is stronger in newborn rats than in older and larger animals subjected to similar

conditions. This question is discussed in greater detail in the paper by Jackson and Stewart ('18).

The number of individual viscera showing an increase in weight during underfeeding is greater in the young rats than at later periods. While most of the individual organs (like the group as a whole) show a decreasing intensity of growth at later periods of underfeeding, there are several exceptions to the rule, as previously noted.

SUMMARY

1. The weights of the various organs, systems and parts of the body were studied in 8 newborn controls and 15 test rats kept at birth weight by underfeeding for periods ending at 11 to 22 days of age.

2. The underfed newborn rats become relatively long-tailed individuals, as previously found in similar experiments on older animals.

3. During maintenance at birth weight the head increases about 45 per cent in weight, compensated for by a corresponding decrease in the trunk and extremities. This is also similar to the results at later stages.

4. The visceral group increases 46 per cent, the integument 25 per cent and the musculature and skeleton (together) 6 per cent in the test rats, counterbalanced by a corresponding decrease of 59 per cent in the "remainder." The integument and visceral group thus show much stronger growth tendencies than in similar underfeeding experiments at later ages. The musculature and skeleton have apparently weaker growth tendencies than later, while the loss in the "remainder" is relatively greater than at later periods.

5. The individual organs in the test rats, as compared with those in newborn rats of corresponding weight show the following percentage increases in weight: testes, 374; epididymis, 225; eyeballs, 146; brain, 125; kidneys, 90; spinal cord, 83; stomach and intestines (empty) 40; spleen, 33; hypophysis, 29; heart, 26; pineal gland, 21; stomach and intestines (with contents), 19.

In the rats kept at birth weight the thyroid gland, ovaries, lungs and suprarenals remain practically unchanged in weight (gain of 8, 5, 5 and 3 per cent respectively); whereas the liver and thymus suffer a loss of 23 and 49 per cent respectively.

6. These changes in rats held at birth weight by underfeeding resemble in general those previously found in somewhat larger and older stunted animals (Jackson, '15b; Stewart, '18a; Jackson and Stewart, '18). Certain differences appear, however, as shown in table 3.

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THE STIMULATION AND INHIBITION OF THE GASTRIC SECRETION WHICH FOLLOWS THE SUBCUTANEOUS ADMINISTRATION OF CERTAIN ORGAN EXTRACTS

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In a previous communication (1) we showed that the subcutaneous injection in dogs of saline extracts of several different organs was followed in each case by an increase in the gastric secretion. Extracts made from the adrenal and pituitary glands alone, of those tested, inhibited the gastric secretion both before and after the injection of the stimulant extracts.

These extracts were made by shaking for two hours the hashed fresh organ in normal salt solution which had been made slightly alkaline with NaOH. This was then stored in the ice box for twenty-four hours. After straining off the coarser particles through gauze and filtering through paper, enough 10 per cent acetic acid was added to the opalescent filtrate to precipitate the nucleo-proteins. After their removal the filtrate was boiled and again filtered to separate the acid albumins and this filtrate rendered faintly alkaline to litmus was, also boiled and filtered. This final slightly alkaline liquid then contained only the non-coagulable portion of the original saline extract and for convenience was designated as the "residue" of this or that organ. The injection into dogs of the "residue" of each of the endocrine glands was followed by some definite and immediate physiological reaction which seemed traceable to either stimulation or inhibition of a part of the involuntary nervous system.

In the case of the thyroid, none of the coagulable materials in its saline extract were found capable of producing any demonstrable reactions.

For the adrenal, however, further experimentation has shown that almost as vigorous responses could be excited in the stomach by the adrenal nucleo-proteins as by the adrenal "residue." The latter is

rich in epinephrin while the nucleo-proteins contain only traces of this substance.

In the following article we have confined ourselves to the study of the effects upon the gastric secretion of the subcutaneous injection of extracts of the pituitary and adrenal glands and more particularly of the thyroid. We had previously employed dogs in which the usual Pawlow gastric pouch and fistula had been established, but as this operation seemed liable to damage the nerve supply of the pouch, we have employed in these experiments the Janeway fistula. A trap door flap about 2 inches square, with its base at the greater curvature, is cut from the anterior surface of the stomach. The flap is turned back and folded together by a continuous suture which unites the right and left borders of the flap and then the right and left edges of the gap in the stomach. The open extremity of the resultant artificial tube, which is continuous with the interior of the stomach, is then brought out through a stab wound in the left rectus and sutured to the skin. This fistula does not leak and after the wounds have healed, readily allows the passage of a catheter for obtaining the gastric secretion.

During the tests the animals have been placed in a retaining frame in which they can be kept quiet for an hour or more. Our dogs were given no food for at least twelve hours before the test. To make them react satisfactorily the animals must be maintained in the best possible condition.

Disease or malnutrition very evidently interferes with the amount of gastric secretion and with the responsiveness of the stomach to the test materials. The thyroid extracts were standardized according to both their iodine and nitrogen content, and the adrenal and pituitary extracts according to their nitrogen content alone. The injections were made with every aseptic precaution, usually into the subcutaneous tissue at the back of the neck. Then, with the dog standing in the retaining frame, a catheter was introduced into the fistula and the gastric secretion collected during successive fifteen-minute periods.

A great number of tests were made and table 1 shows a fair average of the results for the different preparations from the thyroid. It was found to be difficult or impossible to use the same dog for each test because a twenty-four-hour rest period, at the least, had to be allowed to maintain the animals in good condition. This often had to be much extended. The feeding took place in the afternoon, and the morning hours, when the stomach contains no food, were used for the experiments. In the second column of table 1 are noted the

(normal) quantity in cubic centimeters of gastric juice, collected during the first fifteen minutes and its total acidity expressed as HCl. This was determined by titration against $\frac{N}{10}$ NaOH, with phenolphthalein as the indicator. In the next column are noted the thyroid materials and the amount injected expressed in terms of its iodine and protein content. The next three columns represent the quantity and total acidity of the gastric juice collected during the three fifteen-minute intervals which follow the brief period required by the experimenter to make the test injection and then note the time. That is, about five minutes elapse between the close of the collection of the normal flow and the beginning of the collection of the test flow.

As remarked before, we had previously determined that the "residue" of a saline extract of the thyroid of any given animal, when standardized by its iodine content, was quite uniformly active. Of the common abattoir animals, the pig thyroid contains the largest amount of iodine, the sheep contains much less and the beef, the least iodine of the three. Incidentally it should be noted that sheep thyroids are very often goitrous and hence have an extremely low iodine content, and therefore care must be used to select only normal glands. Their iodine content, as is well known, shows a considerable seasonal variation, being the richest during the spring and summer months.

The first test in table 1 shows the comparative activity upon the stomach of the "residue" or non-coagulable portion of saline extracts of the pig, sheep and beef thyroids. The next tests show the comparative activity of pig thyroids, which were obtained from the abattoir in as *fresh* a condition as possible, and immediately extracted, and of those which had been kept before extraction, in a *frozen condition for about two weeks;* and finally, of the activity of an extract or "residue," made in exactly the same way as the others, from the commercial desiccated thyroid powder, which presumably consists of sheep thyroid glands.

As the active material in our "residues" is non-coagulable, we next tested alcoholic and ethereal extracts of the pig thyroid. The hashed glands were shaken for two hours in ether or 95 per cent alcohol, and stored for several days at room temperature, in corked bottles. The alcoholic or ether extract was filtered so that we obtained a clear liquid and then the alcohol or ether distilled off until only about one-eighth to one-tenth of the original volume remained. This was then diluted with distilled water and tested as to iodine and nitrogen content. A similarly prepared 95 per cent alcohol extract of commercial desic-

TABLE 1

Effects upon the flow of gastric juice and its acidity, which follow the injection of thyroid and other "extracts"

DOG NO.	NORMAL FLOW FIRST 15 MINUTES AMOUNT HCl		MATERIAL INJECTED	AMOUNT	EFFECT DURING SECOND 15 MINUTES AMOUNT HCl		THIRD 15 MINUTES AMOUNT HCl		FOURTH 15 MINUTES AMOUNT HCl	
	cc.	*per cent*			*cc.*	*per cent*	*cc.*	*per cent*	*cc.*	*per cent*
XX	3.0	0.46	Pig thyroid residue	7.0 cc. = {0.2 mgm. I; 0.19 gram Prot.	21.5	0.52	21.0	0.54	10.0	0.54
XX	1.5	0.4	Sheep thyroid residue	10.0 cc. = {0.125 mgm. I; 0.25 gram Prot.	6.5	0.52	6.0	0.48	5.0	0.48
XXII	3.4	0.36	Beef thyroid residue	2.8 cc. = {0.028 mgm. I; 0.1 gram Prot.	4.0	0.44	3.0	0.4	2.0	0.4
XXIX	5.0	0.33	Pig thyroid residue "fresh" glands	4.0 cc. = {0.1 mgm. I; 0.4 gram Prot.	13.0	0.33	10.0	0.45	9.0	0.44
XXIX	1.0	0.4	Pig thyroid residue frozen 2 weeks	4.0 cc. = {0.1 mgm. I; 0.4 gram Prot.	12.0	0.5	13.0	0.5	8.0	0.45
VIII	3.4	0.36	Sheep (?) thyroid residue from commercial desiccated powder	2.5 cc. = {0.2 mgm. I; 0.075 gram Prot.	7.0	0.36	6.0	0.38	4.0	0.34
XXIX	4.0	0.5	95 per cent alcohol extract pig thyroid	3.0 cc. = {0.033 mgm. I.; 0.3 gram Prot.	15.0	0.65	16.0	0.6	14.0	0.6
XXIX	2.0	0.36	Ether extract pig thyroid	2.0 cc. = {Trace I; 0.1 gram Prot.	4.0	0.43	5.5	0.46	4.0	0.46

XIV	4.0	0.28	95 per cent alcohol extract desiccated commercial (sheep) thyroid	2.8 cc. =	0.15 mgm. I 0.1 gram Prot.	8.0	0.36	7.0	0.3	7.0	0.3
III	2.6	0.3	Distilled water extract pig thyroid	5.0 cc. =	0.08 mgm. I 0.1 gram Prot.	1.0	0.36	1.0	0.36	Few	drops
VIII	2.6	0.3	Solution of Kendall's preparation from pig thyroids	2.1 cc. =	0.1 gram Prot.	1.5	0.28	1.5	0.28		
III	1.5	0.4	Beef muscle residue	4.0 cc. =	0.1 gram Prot.	1.5	0.4	1.0	0.4		
XXX	Few drops		Edkin's "gastrin"	7.0 cc. =	0.1 gram Prot.	8.0	0.5	11.0	0.58	5.0	0.5

cated thyroid powder (presumably from sheep glands) was also tested. An extract, according to Kendall's method (2) published in 1915, was made and tested. It had a high nitrogen and iodine content.

To show that a "residue" of any tissue is not active there is inserted a test made with the non-coagulable part of a saline extract of beef muscle. In the last line there is recorded for comparison a test made with Edkin's "gastrin" (3).

From the above figures it can be seen that a dose of the pig, sheep or beef thyroid which contains approximately the same amount of protein, varies considerably in the amount of iodine. And the greater the amount of iodine, the greater is the stimulant effect. The "residue" of the pig thyroid increases the gastric flow usually about sevenfold, of the sheep thyroid about fourfold, while the beef gland shows only slight activity. The total acidity is regularly increased but not to any such extent as is the quantity of gastric secretion.

There is a general belief that glands obtained in as "fresh" a condition as possible are preferable to, or more active than, those which have been stored for days or weeks, in a frozen state. In all our tests the frozen glands were fully as active as the "fresh." This is a matter of considerable importance as glands in quantity, for commercial purposes, practically must be stored for a greater or less length of time. The "residue" or alcohol extract of the common desiccated powder of sheep thyroid glands is only about half as active as corresponding preparations from fresh glands. Extracts of the dried material also show an increase in the protein content with a decrease in iodine, and this circumstance combined with its demonstrably lessened activity indicates some deterioration in quality.

An alcohol extract of the thyroid contains a little less iodine and more protein than our "residue" and is somewhat less active, and the average of a number of tests of comparable doses of the alcohol and saline or "residue" extracts shows that their stimulant powers upon the stomach are much in favor of the "residue." An ether extract consists almost entirely of fatty material which for injection has to be made into an emulsion with distilled water. This contains only traces of iodine but shows considerable activity. An ether-alcohol extract shows approximately the same activity as one made with alcohol alone.

The injection of a solution of Kendall's thyroid "active principle," as described above was followed by no response. An extract of pig thyroid made with distilled water was proved by repeated tests to be entirely inert. Thus evidently indicates that hydrolysis is necessary

before the active thyroid material can be obtained in watery solutions. Tests with a "residue" made from beef muscle prove only that hydrolyzed extracts of these organs have no stimulant effect upon the stomach, but the observation is inserted to show that "residues" of all tissues are not gastric activators.

The normal human thyroid contains approximately the same amount of iodine as the pig gland, or about 0.3 mgm. per gram of fresh tissue, and a "residue" or alcohol extract of this is closely comparable in its effects upon the stomach to a similar extract from the pig thyroid. The human organ in an individual who shows the symptoms of hyperthyroidism, is usually enlarged symmetrically, and is of even consistency throughout, in which case the infolding and proliferation of the alveolar epithelium characteristic of its supposed overactivity occurs more or less abundantly according to the severity of the symptoms, and in pronounced cases involves the entire diseased gland. Or it may be asymmetrically enlarged and of uneven consistency, in which case there are usually one or more encapsulated adenomatous or cystic or mixed cyst-adenomatous masses with the characteristic hyperthyroid changes surrounding, but not in the tumors. From these solid adenomata and from typical hyperthyroid glands, obtained fresh from operations, extracts were made as from the pig thyroids and the results recorded in table 2.

It will be noted that the first three tests are recorded from extracts from "human goiter." These were solid adenomata obtained from patients who presented moderate symptoms of hyperthyroidism, but the histology of the excised tissue showed only simple adenoma. The two last tests were each made with extracts of portions of glands containing the characteristic hyperthyroid changes and were obtained from patients suffering from typical exophthalmic goiter. Many other tests were made with extracts from similarly diseased thyroids, but like these, all were uniformly negative. Unfortunately there was seldom enough tissue to make both an iodine and a nitrogen determination, and as these experiments were carried out as material could be obtained, while tests with other organ extracts were in progress, only the protein content of the dose was ascertained. This permitted comparisons with organ extracts other than the thyroid, but there was never any response. That is, the symptoms of hyperthyroidism are apparently not *directly* due to an excessive quantity of thyroid product. Clinically the severity of the disease certainly does not seem to bear any relation to the size of the gland. Neoplastic epithelia are not

TABLE 2

Effects upon the flow of gastric juice and its acidity, which follow the injection of extracts from pathological human thyroids

DOG NO.	NORMAL FLOW FIRST 15 MINUTES AMOUNT HCl		MATERIAL INJECTED	AMOUNT	EFFECTS DURING SECOND 15 MINUTES AMOUNT HCl		THIRD 15 MINUTES AMOUNT HCl		FOURTH 15 MINUTES AMOUNT HCl	
	cc.	*per cent*			*cc.*	*per cent*	*cc.*	*per cent*	*cc.*	*per cent*
XV	3.0	0.21	'Residue' of simple human goiter	2 cc. = 0.19 gram Prot.	2.0	0.24	1.5	0.2	Few drops	
XVIII	8.0	0.27	'Residue' of simple human goiter	7 cc. = 0.1 gram Prot.	5.2	0.36	5.0	0.36	2	0.3
XIII	5.0	0.22	95 per cent alcohol extract simple human goiter	12 cc. = 0.1 gram Prot.	3.6	0.2	2.7	0.2		
XV	4.1	0.18	95 per cent alcohol extract hyperthyroid human gland	10 cc. = 0.4 gram Prot.	2.5	0.36	Few drops			
VIII	8.0	0.37	Residue of human hyperthyroid gland	7 cc. = 0.1 gram Prot.	5.2	0.36	5.0	0.36		

generally believed to produce any secretion and the above experimental findings with thyroid adenomata seem to confirm this belief.

The lack of response from extracts of thyroids which showed active hyperplasia at least indicates so great a change in the quality of the product as to prevent the fulfillment of its usual function.

We next tested the effects upon the stomach of the subcutaneous injection of atropin, nicotine, commercial adrenalin and extracts of the adrenal and pituitary glands. In previous experiments (1) as remarked before, we had ascertained that the adrenal and pituitary were the only organs which seemed capable of inhibiting the gastric secretion. Nicotine was accidentally noted to have this effect and although it is understood to act upon some intermediate nervous mechanism it was employed as a comparison with atropin, which paralyzes the terminal filaments of the vagus.

The records given above are a fair average of a large number of tests. It is well known that the secretory activity of the stomach is very largely controlled by the vagi, stimulation of which increases the flow, and by the sympathetic which inhibits. It can be noted that a moderate dose of atropin very quickly stops the gastric secretion, presumably by paralysis of the terminal filaments of the vagus. Nicotine was tested in both large and minute doses and seemed to exhibit inhibitory powers somewhat less than atropin; the inhibition was about in proportion to the size of the dose. Certainly no stimulation could be detected after the administration of even very minute amounts.

Epinephrin is generally accepted as acting through some stimulant effect upon the terminal filaments of the sympathetic, and thus should inhibit the gastric secretion. The typical test with a 1:1000 solution of adrenalin hydrochloride recorded in the third line of table 3, shows that a moderate dose cuts down the normal flow of gastric juice about one-half. A similar dose of a "residue" or an alcohol extract of the adrenal, which contains considerably more epinephrin, suggests that these materials, because of their epinephrin content, act in proportion to it by stimulation of the terminal filaments of the sympathetic. But the injection of a comparable quantity of the adrenal nucleo-proteins, which contain only faint traces of epinephrin is as active or more so than the adrenal "residue" or the alcohol extract or commercial adrenalin. Presumably its effects upon the secreting gastric epithelium is like adrenalin and exerted through the intermediation of the terminal sympathetic filaments. But if it is even more active than adrenalin this fact arouses a suspicion that the active principle of the adrenal

gland may contain something more than epinephrin. That is, epinephrin which undoubtedly exists in the adrenal, may be only the stable part of a larger and more labile molecule.

The final three tests with extracts, first of the whole pituitary gland and then with similar preparations from its anterior and posterior lobes, show only that the anterior lobe of this gland seems to exert some inhibitory influence upon the gastric secretion.

TABLE 3

Effects upon the flow of gastric juice and its acidity, which follow the injection of atropin, extracts of the adrenal and pituitary glands, and of nicotine

DOG NO.	NORMAL FLOW FIRST 15 MINUTES AMOUNT HCl		MATERIAL INJECTED	AMOUNT	FLOW DURING SECOND 15 MINUTES AMOUNT HCl		THIRD 15 MINUTES AMOUNT HCl	
	cc.	*per cent*			*cc.*	*per cent*	*cc.*	*per cent*
VIII	4.1	0.3	Atropin sulf.	15 mgm.	0.5	0.4	Few	drops
VII	2.2	0.2	Nicotine	1 mgm.	Few	drops	Few	drops
IX	6.0	0.65	1:1000 solution adrenalin chloride	3 cc.	3.0	0.65	2.0	0.65
VIII	3.1	0.33	Adrenal "residue"	3.0 cc. = 0.1 gram Prot	0.2	0.35	Few	drops
XI	2.6	0.53	95 per cent alcohol extract adrenal	6.3 cc. = 0.076 gram Prot.	0.5	0.44	0.15	0.63
XI	4.0	0.28	Adrenal nucleoprotein	4.75 cc. = 0.076 gram Prot.	0.1	?	Few	drops
XI	2.4	0.38	Pituitary residue	1.26 cc. = 0.076 gram Prot.	1.0	0.36	0.9	0.38
VI	2.6	0.19	Residue anterior lobe pituitary	2.75 cc = 0.076 gram Prot.	0.9	0.14	0.8	0.1
V	1.5	0.3	Residue post. lobe pituitary	3.4 cc = 0.1 gram Prot.	1.8	0.35	1.4	0.37

In table 4 are recorded the average results of a large number of tests to show first the stimulant effect which follows the injection of the thyroid "residue," and the effect upon this stimulation which follows the injection of *a*, atropin to paralyze the vagus; *b*, of adrenalin to stimulate the antagonist of the vagus or the sympathetic; *c*, of the adrenal "residue" which contains much epinephrin and presumably acts like the 1:1000 solution of adrenalin; *d*, of the alcohol extract of

TABLE 4

Effects which follow the injection of atropin, extracts of the adrenal and pituitary glands, and nicotine, after the injection of thyroid material has stimulated the flow of gastric secretion

DOG NO.	NORMAL FLOW FIRST 15 MINUTES AMOUNT HCl		STIMULATION BY	AMOUNT INJECTED	FLOW DURING SECOND 15 MINUTES AMOUNT HCl		INHIBITION BY	AMOUNT	FLOW DURING THIRD 15 MINUTES AMOUNT HCl		FOURTH 15 MINUTES AMOUNT HCl		LINE NO.
	cc.	*per cent*			*cc.*	*per cent*			*cc.*	*per cent*	*cc.*	*per cent*	
VIII	4.0	0.25	Thyroid residue	5.2 cc.=0.1 gram Prot.	17.0	0.38	Atropin sulf.	15 mgm.	6.0	0.38	2.2	0.3	1
XVIII	2.0	0.18	Thyroid residue	4.0 cc.=0.1 gram Prot.	16.0	0.27	Atropin sulf	15 mgm.	9.4	0.42	9.0	0.48	2
XVII	1.5	0.12	Thyroid residue	4.0 cc.=0.1 gram Prot.	8.0	0.2	1:1000 solution adrenalin chloride	1.5 cc	5.0	0.3	1.0	0.36	3
III	1.0	0.36	Thyroid residue	3.7 cc.=0.1 gram Prot.	12.0	0.55	Adrenal residue	3.0 cc.=0.1 gram Prot.	1.1	0.53	4.8	0.53	4
I	1.4	0.36	Thyroid residue	3.7 cc.=0.1 gram Prot.	7.5	0.47	Adrenal nucleoproteins	6.2 cc.=0.1 gram Prot.	1.2	0.42	0.15	0.32	5
III	0.2	0.2	Thyroid residue	3.7 cc.=0.1 gram Prot.	4.6	0.25	95 per cent alcohol extract adrenal	8.3 cc.=0.1 gram Prot.	1.6	0.21	1.8	0.27	6
VII	0.2	0.2	95 per cent alcohol extract thyroid	2.0 cc.=0.1 gram Prot.	7.4	0.24	Pituitary residue	2.0 cc.=0.1 gram Prot.	4.0	0.21	3.0	0.27	7
VIII	4.8	0.28	Thyroid residue	4.0 cc.=0.1 gram Prot.	16.2	0.38	Nicotine	0.75 gram	11.0	0.36	7.5	0.38	8

the adrenal which is closely similar to the adrenal "residue;" *e*, of a solution of the adrenal nucleo-proteins which contains only traces of epinephrin; *f*, of the pituitary "residue," and finally *g*, of nicotine.

After stimulation of the gastric flow by the injection of the non-coagulable or "residue" part of a thyroid extract, paralysis of the vagus by atropin only checks the secretion. Atropin does not then completely stop the flow as it does in the unstimulated stomach. Adrenalin, on the other hand, which "activates" the sympathetic or inhibitory nerve supply of the stomach, only checks the *unstimulated* flow, and shows about the same amount of inhibition upon the stimulated secretion. The injection of the adrenal "residue" or of an alcohol extract of the adrenal, which are both rich in epinephrin, is followed by about the same decrease in the flow of the unstimulated, but a considerably greater decrease in the stimulated gastric secretion.

The injection of a solution of the adrenal nucleo-proteins which contains only traces of epinephrin, is followed by a similar and even a slightly more vigorous inhibition. These facts seem to indicate that the gastric nerves are very important, if not essential, for the stimulation produced by the thyroid and for the inhibition by the adrenal. Apparently also the thyroid acts through or upon the vagus and not, as is generally believed, upon the sympathetic. The inhibitory powers of the latter nerves are apparently intensified by some substance produced by the adrenal gland, in which epinephrin forms a part. This substance is probably not epinephrin alone, because adrenalin does not act as vigorously as do extracts of the whole adrenal gland, or the adrenal nucleo-proteins, in which, as stated previously, there are only traces of epinephrin (table 4, lines 3, 4 and 5).

The non-coagulable or "residue" part of a saline extract of the pituitary is the only other organ derivative which we have found to possess inhibitory powers over the flow of gastric secretion. It is interesting to note that extracts of the posterior lobe seem inert. The anterior only is active, but an extract of the whole gland proved more active than one of the anterior lobe alone. Nicotine is supposed to paralyze not the terminal filaments of the sympathetic, but an intermediate portion of the system. It was employed in these tests because it was unexpectedly found to inhibit the gastric secretion in more or less direct proportion to the size of the dose.

As yet we can offer no very definite explanation for these phenomena. Atropin quite evidently paralyzes and so stops the functionation of the "activating" or autonomic portion of the involuntary nervous system.

It is also evident that the adrenal product has a selective affinity for the terminal filaments of the sympathetic, as distinguished from the autonomic or vagus group of nerves. The thyroid product, on the other hand, seems to possess at least some of the same kind of affinity or stimulant power for the opposing or vagus group of filaments. It certainly does not stimulate the sympathetic, or its effect upon the stomach would be intensified and not decreased by the injection of either atropin or adrenalin.

Ergotin is supposed to paralyze the terminal filaments of the sympathetic, but in our experiments upon the stomach it was found to be so variable and uncertain as to be useless. Our extracts of the whole adrenal gland all contain at least traces of epinephrin, and therefore presumably act like it, i.e., through or upon the sympathetic. This, of course, brings into question epinephrin. Is it the normal active principle of the gland or only the stabile portion of a larger and more labile product? That cannot be answered from these experiments, although it is quite evident that extracts of the whole gland show a greater inhibitory effect upon the gastric secretion than does adrenalin.

DISCUSSION

The thyroid gland evidently stimulates, and the adrenal inhibits, gastric secretion. These effects are apparently produced chiefly through the intermediation of the vagus and sympathetic nerve terminals. The secretory functions of the vagus are apparently excited by some material which can be extracted from the thyroid by alcohol or by a process which involves more or less hydrolysis of the gland. The inhibitory powers possessed by the sympathetic terminals seem to be excited by all the extracts from the adrenal gland which were tested. Extracts from the entire gland are much more active than adrenalin. Can this activation or inhibition of the secretory cells of the stomach through their nerve supply in conjunction with thyroid and adrenal products be a process which is essentially one of nutrition? A prolonged stimulation like that produced by the thyroid extracts, when compared with the increased general metabolism which follows thyroid feeding, suggests that the thyroid product, with the intermediation of the vagus or secretory nerve impulse, increases the metabolism of the gastric epithelium. This means that it facilitates the absorption of nutritional material or "food" by the cells and the metamorphosis of this "food" into the cell's secretion. The adrenal product, on the other

hand, in conjunction with the sympathetic or inhibitory nerve impulse, can be imagined as preventing this metamorphosis. The nature of the impulses conveyed to the stomach by its double nerve supply is, of course, unknown but, as determined by electrical stimulation, the vagus evidently activates and the sympathetic inhibits this organ If the thyroid product has an affinity for the vagus terminals and promotes cell metabolism, and the adrenal an affinity for the sympathetic and retards this metabolism, then it is unnecessary to imagine two radically different kinds of nerve impulses. The chemical properties of the nerve endings are alone to be considered. The nature of the impulse in the vagus and the sympathetic can be the same, but the effects of its discharge from one nerve terminal or the other are regulated by the presence and amount of thyroid or adrenal, and probably many other products.

CONCLUSIONS

1. A slightly alkaline saline solution, or alcohol, extracts from the thyroid some non-coagulable material which is a vigorous stimulant for the gastric secretion.

2. This material produces its effects at least in part by intensification of the functions performed by the terminal filaments of the (gastric) vagus.

3. Extracts similarly made from the pathological tissue of adenomatous or hyperthyroid human glands are inert.

4. Extracts of the adrenal gland vigorously inhibit gastric secretion.

5. These extracts of the adrenal gland all contain more or less epinephrin and therefore it is presumable that their effect is produced by intensification of the inhibitory function which is ascribed to the (gastric) sympathetic.

6. Adrenalin is not as active a gastric inhibitor as the adrenal nucleoproteins obtained from extracts of the whole gland. These adrenal nucleo-proteins contain only traces of epinephrin.

7. Extracts of the pituitary gland also inhibit gastric secretion, but only about one-half as vigorously as do extracts of the adrenal.

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QUANTITATIVE STUDIES ON THE RATE OF RESPIRATORY METABOLISM IN PLANARIA

I. The Effect of Potassium Cyanide on the Rate of Oxygen Consumption

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INTRODUCTION

For the study of energy relations in organisms, the lower animals offer certain advantages over the higher vertebrates. Such regulatory mechanisms as those controlling the ventilation of the lungs, the rate of flow of the blood, the oxygen-carrying power of the blood and the maintenance of constant body temperature, which impose limitations upon experimentation with higher vertebrates, can be avoided by the selection of proper material from among invertebrates. Planaria possess none of these mechanisms and they are well fitted for such studies because they can withstand prolonged starvation or can ingest a large meal, and they have remarkable powers of regeneration and agamic reproduction. Experiments upon Planaria have played a prominent rôle in the formulation of theories by Child (1) regarding the so-called "rate of metabolism" in relation to senescence, rejuvenescence and dominance in organisms. Child used the susceptibility of animals and parts of animals to the toxic action of KCN as a measure of the "rate of metabolism." This use of cyanide was based upon the belief of many physiologists that it inhibits oxidations in organisms and upon certain qualitative tests in which, it is reported, the carbon dioxide production by worms in Tashiro's "biometer" agreed with what was expected from their susceptibility to cyanide. No quantitative studies of the metabolism of Planaria by direct methods of measurement have been published heretofore. The author has undertaken a series of such studies for the purpose of determining whether KCN can be used to measure the rate of oxidation in these animals and to determine the

important facts regarding the rate of oxidation in Planaria in relation to nutrition, age and morphogenesis. These studies were suggested by Prof. E. J. Lund, under whose direction they have been carried out and to whom I wish to express my most sincere appreciation for assistance and encouragement.

The present paper deals with methods of measuring the oxygen consumption by Planaria and the effect of KCN upon the oxygen consumption by these animals.

The belief that cyanides inhibit intracellular oxidations finds support in a number of studies of the respiratory exchange of organisms, beginning with the classical studies of Geppert (2). Schroeder (3) reported a marked decrease in the oxygen consumption by the mold, Aspergillus niger, in KCN solutions. Warburg (4) and Loeb and Wasteneys (5) agree that the oxygen consumption by sea urchin eggs is decreased very strongly in cyanide solutions; and Loeb and Wasteneys (6) have shown a similar effect of cyanide on the oxygen consumption by Fundulus embryos and by the jellyfish, Gonionemus. Experiments of Hyman (7) also tend to show a decrease in the oxygen consumption by marine sponges in KCN, although there may be question regarding the rate of flow of water through the sponge in cyanide solutions in these experiments. On the other hand Lund (8) has found that the oxygen consumption by Paramecium is independent of the action of KCN.

In non-living systems various authors since Schonbein (9) have found that cyanides inhibit the activity of oxidizing enzymes, but in some cases the cyanide has an opposite effect. Thus Kastle and Loevenhart (10) report that HCN inhibits the oxidations of guaiacum by potato oxidase and many inorganic oxides and peroxides, but that it accelerates the oxidation of guaiacum by silver oxide. Loevenhart (11) found that HCN retards catalysis of hydrogen peroxide by platinum black, silver and extract of tissues, but that it accelerates catalysis of hydrogen peroxide when copper or iron and its salts are used. And the same author (12) finds that HCN accelerates the catalyzing action of copper sulphate upon the oxidation of formic acid by hydrogen peroxide. Matthews and Walker (13) find that HCN prevents the oxidation of cystein to cystin but (14) that it accelerates the oxidation of impure cystin or cystin solutions containing ferric chloride, and that it has no effect on the oxidation of pure cystin.

These results lead to caution about predicting that KCN inhibits oxidations in every system. Are the oxidation systems in all organisms

alike in respect to KCN or are there some oxidation systems like the oxidation of cystein and other systems like the oxidation of cystin? May there be other animals than Paramecium whose respiratory exchange is not affected by cyanides? In determining the value of KCN as a measure of the rate of oxidations in any form it is necessary, first of all, to determine its effect upon the oxidations in that form. It has not been shown, heretofore, that KCN affects the oxidations in Planaria.

METHODS

The materials used in this investigation were Planaria maculata, collected from the Mississippi River, and Planaria agilis, purchased and reared in the laboratory. The latter species has been found more convenient for experimentation and most of the experiments reported in this paper were performed with this species. Worms of the former species cling to the substratum so tenaciously that it is often necessary to loosen them with a probe. Not only is this inconvenient but it introduces the danger of injuring the animals.

In each experiment the animals selected were as nearly uniform in size as possible, and had not recently regenerated the head. All animals used in a single experiment were taken from the same stock, having the same history for some days previous to the experiment. The nutritive history and the relative size of worms used in each experiment are stated in the heading of the table reporting the results of the experiment.

The general method employed for determining the oxygen consumption was to place worms in tap water of known content of dissolved oxygen, and at the end of a stated period of time to remove the worms and analyze the water for dissolved oxygen, and so to determine by difference the amount of oxygen consumed. The general procedure was similar to that described by Lund (15) for determining the oxygen consumption by Paramecium, except that the worms were removed from the bottles at the end of the experiment, before the reagents were added, thus eliminating any errors due to absorption of iodine by the organisms during the analysis.

Reagents for Winkler's method of analysis were made up as given by Sutton (16, p. 285) except that 2 cc. of concentrated hydrochloric acid were used as given by Birge and Juday (17, p. 13). The thiosulphate solution was standardized at intervals against known weights of freshly resublimed iodine.

A large carboy was filled with the tap water to be used in an experiment. When the tap water was much warmer or cooler than the room temperature it was allowed to stand in the carboy for twenty-four hours or longer to reach the room temperature. The water was thoroughly stirred and brought into approximate equilibrium with room air as to its dissolved oxygen by drawing a stream of air through it for about two hours. The bottles used in the experiment were then all filled in rapid succession by siphoning water from the carboy to the bottom of the bottles, allowing them to overflow about three times their volume of water. All bottles had fitted ground glass stoppers and were of the same capacity. Bottles which had originally 1 or 2 cc. more than 132 cc. capacity contained pieces of glass rod to

TABLE 1

Showing the degree of uniformity of filling and analysis of 28 bottles filled in order with aerated tap water and analyzed at once

CC. OF THIOSULPHATE	CC. OF THIOSULPHATE	CC. OF THIOSULPHATE	CC. OF THIOSULPHATE
4.55	4.56	4.53	4.58
4.59	4.55	4.57	*4.52*
4.56	4.56	*4.60*	4.55
4.52	4.54	4.58	4.59
4.57	4.55	4.58	4.56
4.58	4.56	*4.52*	4.57
4.57	4.55	4.56	4.53

Maximum..... 4.60 cc. thiosulphate
Minimum..... 4.52 cc. thiosulphate
Average..... 4.56 cc. thiosulphate

make them uniform with the others. The degree of uniformity of filling and analysis is illustrated by table 1, which shows the result of filling the whole series of 28 bottles with aerated tap water and analyzing them at once. The analyses ranged from 4.52 cc. to 4.60 cc. thiosulphate. The average of all analyses was 4.56 cc. thiosulphate. The maximum error in an individual analysis, therefore, was about 1 per cent of the total oxygen content of a bottle, and since duplicate determinations were made in all experiments, the error in filling and analysis was reduced even below this figure.

The bottles needed in an experiment were filled in order, as explained above. Then three or four bottles, selected from different parts of the series, were set aside as "blanks." For example, in the experiment reported in table 2 the 1st, 6th and 11th bottles filled were chosen as

blanks. The average of the analyses of the blanks was taken to represent the amount of oxygen in each of the other bottles at the beginning of the experiment. Uniformity among blanks, chosen in this way, was taken to show uniformity of filling of the whole series of experimental bottles.

Worms were introduced into experimental bottles by means of a glass pipette of suitable size. With care, a worm can be introduced into a bottle by touching the pipette to the surface of the water in the bottle without introducing more than a drop of water. In removing worms at the end of an experiment, the Planaria agilis were loosened by gently rotating the bottle, and were then picked out individually by means of the pipette. In this operation, care was taken not to introduce any bubbles of air in the bottle and not to draw into the pipette any water from the vessel into which the worms were transferred. Planaria maculata cling to the substratum very tenaciously. Frequently it was necessary to loosen them by means of a wire probe, flattened at the end to form a spatula. The following tests show that the error from introducing probes and pipettes into bottles to remove worms was negligible. Seven bottles were filled with aerated tap water. Four of these were analyzed as controls, giving an average oxygen content of 4.03 cc. thiosulphate. Each of the other three bottles was opened and the pipette was introduced twenty-five times, drawing in and expelling water thrice each time. The average oxygen content of these bottles was then found to be 4.04 cc. thiosulphate, showing no difference from the controls. The water in the bottles in this case was approximately in equilibrium with air as to its dissolved oxygen. Under such circumstances it was not to be expected that the manipulations described would affect the oxygen content appreciably.

At the end of an experiment, however, after part of the oxygen has been consumed, the water is no longer in equilibrium with air and these manipulations might alter the oxygen content. The following test meets these conditions. Six bottles were filled with boiled tap water of low oxygen content. Three alternate bottles were analyzed as controls, giving an average oxygen content of 1.74 cc. thiosulphate. Into each of the other three bottles ten worms were introduced and then immediately removed. These experimental bottles gave an average oxygen content of 1.80 cc. thiosulphate, showing a difference of 0.06 cc. thiosulphate from the controls. In the experiments to be reported, the oxygen content of the bottles at the end of the experimental periods was rarely reduced below 2.50 cc. thiosulphate. More-

over, any error from this source would tend to diminish differences in oxygen consumption rather than to produce them to any degree.

In some cases, removing worms removed so much water that there was danger of enclosing a bubble of air in closing the bottle after introducing the reagents. To prevent this possibility, the uniform procedure was followed of displacing 2 cc. of water with a glass rod before introducing the reagents. The rods used for this purpose were of the same volume and were marked so that they could not be confused with other rods present in certain bottles for the purpose of standardizing the volume. This procedure was adopted in experiments with Planaria maculata, which are difficult to handle with a pipette, and was continued in a routine manner in all experiments. In all cases blanks were handled in the same manner as experimental bottles in this respect. In calculating the oxygen consumption in cubic centimeters of oxygen gas, cf. table 10, columns 3 and 6, correction was made for the 4 cc. displaced by the glass rods as well as for the 2 cc. displaced by the added reagents. These corrections were not made in the tables giving the oxygen consumption in cubic centimeters of thiosulphate since they do not alter relative values.

It required from two to four minutes to remove worms from a bottle and introduce the reagents. When an experiment contained several parts and there were two or three duplicate bottles in each part, it often required half an hour or even an hour to carry out these manipulations. Under such circumstances, to avoid serious error from the fact that the length of the experimental period was greater for certain bottles than for others, bottles were handled in the same order at the end of the period as at the beginning. In all experiments a record was made of the exact time of handling each bottle, and these times were so arranged as to insure that there was no variation from bottle to bottle in the length of the experimental period.

The following test shows that the iodine error from mucous secretions of the worms was also negligible. In each of four bottles, six worms were left for four and a half days, after which time they were removed and the bottles were filled slowly with aerated tap water so as to avoid violent currents that might remove accumulations of mucus. The average of the four analyses was 4.63 cc. thiosulphate, while four clean bottles filled at the same time from the same aerated tap water gave an average analysis of 4.65 cc. thiosulphate.

In the experiments reported in the present paper, each contains its own temperature control since all comparisons that have been made

are between worms that were tested simultaneously and under the same conditions of temperature. However, except in the first two preliminary experiments, all bottles were placed in a water bath within a constant temperature oven at 20°C., so that different experiments are comparable so far as temperature is concerned.

For each experiment the potassium cyanide was freshly weighed out, and a relatively strong solution, e.g., 0.01 molecular, was made with the aerated tap water used in the experiment. The bottles were filled in the usual manner. Those bottles that were not to contain cyanide were closed at once. A measured amount of the stronger cyanide solution was run in from a pipette or burette at the bottom of the other bottles, which were then closed and shaken well before introducing worms. The concentration of the solution was regulated so that the amount of solution added never exceeded 5 cc. and was usually about 2 cc. Upon opening the bottles, before introducing worms, a 2 cc. rod was inserted into each, displacing enough water to make it certain that it could be closed again without leaving any bubbles of air. All bottles in the experiment, including the blanks, were handled alike in this respect.

Potassium cyanide in the concentrations used does not cause any appreciable error in the Winkler oxygen analysis. This was shown by a preliminary test in which three bottles of aerated tap water containing KCN in 0.0004 molecular concentration gave an average oxygen analysis of 4.00 cc. thiosulphate, while three bottles of aerated tap water without cyanide gave an average of 3.99 cc. thiosulphate. In some experiments cyanide solution was introduced into part of the blanks but no appreciable difference in the analyses was found between them and the other blanks not containing KCN, so that this procedure was not always followed.

The experimental method has been explained in considerable detail because several sources of error might readily occur if care is not taken in the details of manipulation. However, when careful methods are followed, their accuracy may be judged by their results. When duplicate determinations in the same experiment show uniformity of result and when a series of determinations with graduated differences in a certain experimental factor such as concentration of KCN shows a consistently graduated series of differences in result, the experiment carries conviction of reliability even when the actual figures are not large. All experiments have been carried out in duplicate of two or three simultaneous but independent determinations as a control upon

the accuracy of the methods. The tables show all the individual determinations, even at the expense of making the tables more involved, in order that the uniformity among duplicate determinations may be clearly seen.

OXYGEN CONSUMPTION BY PLANARIA IN KCN SOLUTIONS OF DIFFERENT CONCENTRATIONS

To determine, first of all, whether KCN decreases the oxidations in Planaria, a preliminary experiment was performed with Planaria maculata, in which the oxygen consumption was tested first in aerated tap water and next in aerated tap water containing KCN. Table 2 states the conditions and gives the results. The headings of the columns are self explanatory. Part A gives the analyses of the blanks, which tell the amount of oxygen in the experimental bottles of part B at the beginning of the experiment, and which serve also as a control for the uniformity of filling of the experimental bottles. The first test in aerated tap water is a control to show the degree of uniformity of oxygen consumption by the animals used in the different bottles of the experiment. In the second test, the bottles of set I contained no KCN, and serve, therefore, as a control for those sets in which cyanide was used. Bottles of set II contained the strongest KCN solution, set III a weaker solution and set IV the most dilute solution. Column 4 shows that in every case worms in cyanide solutions used much less oxygen than those in pure water. In the previous test, column 2, they all used approximately the same amounts of oxygen. Column 4 indicates also that worms in the weakest solutions, i.e., set IV, used more oxygen than those in stronger solutions, but the worms in the stronger solutions suffered injury, as is shown in column 6, so that this experiment is inconclusive so far as concerns the relative effects of KCN solutions of different concentrations. Worms of set IV, however, all of which remained alive and uninjured in 0.000076 molecular KCN, showed a reduction of over half in their oxygen consumption.

A second preliminary experiment was then performed with Planaria agilis, using weaker solutions of KCN. The conditions and results are shown in table 3. The general organization of the experiment and the controls was the same as in the former experiment. Column 2 shows again that worms in cyanide solutions invariably consumed less oxygen than those in pure water, while column 1 shows that in the preliminary test in pure water they all consumed approximately the

TABLE 2

Preliminary experiment showing decrease in the oxygen consumption by Planaria maculata in KCN solutions. Worms, medium size, in laboratory six days without food, 10 individuals in each bottle. Room temperature 20° to 24°C. 1 cc. thiosulphate = 0.1751 cc. oxygen at N. T. P.

PART	SET	BOTTLE	FIRST TEST (CONTROL) (DURATION 12.13 HOURS)			SECOND TEST (DURATION 10.5 HOURS)				
			Blanks			Blanks				
			cc. thio.			*cc. thio.*				
A	Blanks (no worms present)	1	3.36			3.90				
		2	3.32			3.95				
		3	3.33			3.91				
	Average		3.34			3.92				
			(1)	(2)		(3)	(4)		(5)	(6)
			Oxygen remaining at end of period	Oxygen consumed		Oxygen remaining at end of period	Oxygen consumed		Concentration of KCN in each bottle	Condition of worms at end of test
			cc. thio.	*cc. thio.*	*average*	*cc. thio.*	*cc. thio.*	*average*		
B	I	1	2.69	0.68		3.33	0.62			
		2	2.65	0.69	0.67	3.29	0.63	0.64	No KCN	All alive
		3	2.70	0.64		3.23	0.66			
	II	1	2.64	0.70		3.73	0.19			3 disintegrating
					0.70			0.19	0.00015 M	2 curled up
		2				3.74	0.18			2 disintegrating
										4 curled up
	III	1	2.69	0.65		3.80	0.12			4 disintegrating
					0.67			0.15	0.000114 M	1 curled up
		2	2.65	0.69		3.74	0.18			4 disintegrating
										2 curled up
	IV	1	2.78	0.56		3.70	0.22			
					0.61			0.24	0.000076 M	All alive
		2	2.69	0.65		3.66	0.26			

same amounts. In this experiment all worms remained alive and uninjured. A comparison of the averages in columns 2 and 4 shows that there is an inverse relation between the concentration of the cyanide and the oxygen consumption or, in other words, that there is

TABLE 3

Preliminary experiment showing decrease in the oxygen consumption by Planaria agilis in KCN solutions. Worms, 11 to 13 mm. in length; fed beef liver daily till three days previous to the experiment; 10 individuals in each bottle. Each test period of 8 hours duration. Room temperature, 22° to 24°C. 1 cc. thiosulphate = 0.1751 cc. oxygen at N. T. P.

PART	SET	BOTTLE	FIRST TEST (CONTROL) Blanks		SECOND TEST Blanks		
			cc. thio.	*average*	*cc. thio.*	*average*	
A	Blanks (no worms present)	1	3.80	*3.79*	3.74	*3.73*	No KCN
		2	3.80		3.69		No KCN
		3	3.81		3.75		0.000038 Mol. KCN
		4	3.76		3.73		0.000038 Mol. KCN

PART	SET	BOTTLE	(1) Oxygen consumed (Control)		(2) Oxygen consumed		(3) Average rate of oxygen consumption in per cent of normal	(4) Concentration of KCN in each bottle	(5) Condition of worms at end of test
			cc. thio.	*average*	*cc. thio.*	*average*	*per cent*		
B	I	1	0.52	*0.52*	0.38	*0.40*	(Normal)	No KCN	All alive
		2	0.51		0.42				
	II	1	0.47	*0.49*	0.14	*0.15*	*38*	0.000076M	All alive
		2	0.50		0.16				
	III	1	0.56	*0.51*	0.27	*0.24*	*60*	0.000038M	All alive
		2	0.45		0.21				
	IV	1	0.52	*0.52*	0.35	*0.35*	*88*	0.0000076M	All alive
		2	0.52		0.35				

a direct relation between the concentration of KCN and the decrease in the oxygen consumption. The averages of column 2 have been converted into percentages in column 3, where it is seen that the oxygen consumption in 0.000076 molecular KCN was only 38 per cent of

normal. In the previous experiment, table 2, the oxygen consumption in this same concentration of cyanide was 36 per cent of normal.

It might be noted that at the end of this experiment, when the worms in cyanide were disturbed, they all became active but there was a very marked gradation in the rate of locomotion in the different concentrations of KCN. In the strongest cyanide the rate was least while in the weakest cyanide the rate was only slightly less than that of the worms in pure water.

In order to determine the general character of the proportionality between the concentration of the cyanide and the amount of oxygen consumed, the experiment summarized in table 4 was performed. The general plan of the experiment was the same as that of the previous experiments except that a greater number and a wider range of concentrations of KCN were used. Seven different concentrations were employed, ranging from 0.00000076 molecular to 0.00038 molecular. Column 1 shows a high degree of uniformity in the oxygen consumption of the different sets in the first test without cyanide. Column 2 shows that in the second test the oxygen consumption decreased very regularly as the concentration of KCN increased. In the strongest solution, set VIII, the worms were all disintegrated at the end of the experimental period so that the figures in this part of the table may, perhaps, be neglected. Worms in sets VI and VII seemed to be near the limit of their resistance and the figures of set VII might be questioned.

While the experiment recorded in table 4 gave very consistent results, the absolute values were so small that the experiment was repeated with larger worms, as summarized in table 5. The "second part" of this experiment will be discussed under the topic "Reversibility of the action of KCN on Planaria." For the present, attention is confined to the "first part," i.e., to the first and second tests. In this experiment all worms remained alive and uninjured. In the control test, the worms in each of the different bottles consumed approximately the same amount of oxygen, column 1. In the cyanide test the worms in 0.00023 molecular KCN, set VII, consumed only 28 per cent as much oxygen as the control, set I. Worms in 0.00015 molecular KCN consumed 35 per cent as much as the control and worms in 0.000076 molecular KCN consumed 53 per cent as much oxygen as the control. In the still weaker concentrations the oxygen consumption was practically the same as the control. These concentrations were very dilute and were chosen to see whether minimal

TABLE 4

Showing the relation of decrease in the oxygen consumption by Planaria agilis in KCN solutions to the concentration of the KCN. Worms, 10 to 13 mm. in length; fed beef liver almost daily till 12 days previous to experiment; 10 individuals in each bottle. Temperature 19.7° to 19.8°C. 1 cc. thiosulphate = 0.1751 cc. oxygen at N. T. P.

PART	SET	BOTTLE	FIRST TEST (CONTROL) (DURATION 12 HOURS)		SECOND TEST (DURATION 15 HOURS)				
			Blanks		Blanks				
			cc. thio.	average	cc. thio.			average	
A	Blanks (no worms present)	1	4.05	4.06	4.17			4.17	
		2	4.08		4.17				
		3	4.07		4.17				
		4	4.04		4.15				
			(1)		(2)		(3)	(4)	(5)
			Oxygen consumed (Control)		Oxygen consumed		Average rate of oxygen consumption in per cent of normal	Concentration of KCN in each bottle	Condition of worms at end of test
			cc. thio.	average	cc. thio.	average	per cent		
B	I	1	0.30	0.29	0.39	0.39	(Normal)	No KCN	All alive
		2	0.27		0.39				
	II	1	0.30	0.32	0.36	0.36	92	0.00000076M	All alive
		2	0.33		0.36				
	III	1	0.33	0.32	0.38	0.35	90	0.0000076M	All alive
		2	0.30		0.32				
	IV	1	0.32	0.32	0.25	0.24	62	0.000038M	All alive
		2	0.32		0.22				
	V	1	0.32	0.34	0.18	0.20	51	0.000076M	All alive
		2	0.35		0.21				
	VI	1	0.33	0.30	0.15	0.15	38	0.000114M	Slightly injured
		2	0.27		0.15				Slightly injured
	VII	1	0.35	0.33	0.17	0.13	33	0.00015M	Injured
		2	0.31		0.09				Injured
	VIII	1	0.33	0.31	0.11	0.09	23	0.00038M	All disintegrated
		2	0.28		0 06				All disintegrated

TABLE 5

First part (first and second tests) showing the relation of the decrease in the oxygen consumption by Planaria agilis in KCN solutions to the concentration of KCN. Worms, 15 to 16 mm. in length and very broad, without food for 28 days previous to experiments, 10 individuals in each bottle. Each test period of 12 hours duration, with 12 hours interval between tests. Temperature 19.5° to 20.0°C. 1 cc. thiosulphate = 0.1729 cc. oxygen at N. T. P.

Second part (third test) showing reversibility of the effect of KCN in reducing the oxygen consumption. Third test follows second test after interval of 12 hours.

PART	SET	BOTTLE	FIRST PART				SECOND PART	
			First test (Control)		Second test		Third test	
			Blanks		Blanks		Blanks	
			cc. thio.	*average*	*cc. thio.*	*average*	*cc. thio.*	*average*
	Blanks (no worms present)	1	4.39		4.38		4.34	
		2	4.41	*4.40*	4.38	*4.36*	4.31	*4.31*
		3	4.39		4.33		4.29	
		4	4.40		4.36		4.31	

			(1)		(2)		(3)	(5)	(6)	
			Oxygen consumed (Control)		Oxygen consumed		Average rate of oxygen consumption in per cent of normal	Concentration of KCN in each bottle	Oxygen consumed	
			cc. thio.	*average*	*cc. thio.*	*average*	*per cent*		*cc. thio.*	*average*
B	I	1	1.00		0.99				1.04	
		2	1.03	*1.02*	0.93	*0.97*	(Normal)	No KCN	0.96	*1.00*
		3	1.02		0.99				0.99	
	II	1	1.10		1.01				1.03	
		2	1.10	*1.07*	1.05	*1.01*	*104*	0.00000015M	1.01	*1.00*
		3	1.02		0.98				0.96	
	III	1	1.05		1.01				1.01	
		2	1.03	*1.03*	0.99	*1.00*	*103*	0.00000076M	0.98	*0.98*
		3	1.00		1.00				0.95	
	IV	1	1.05		0.94				1.01	
		2	1.01	*1.05*	0.96	*0.98*	*101*	0.0000076M	1.01	*1.03*
		3	1.08		1.04				1.06	
	V	1	0.90		0.51				0.96	
		2	1.03	*0.98*	0.50	*0.51*	*53*	0.000076M	1.01	*0.99*
		3	1.02		0.53				1.01	
	VI	1	1.04		0.32				1.01	
		2	1.11	*1.04*	0.36	*0.34*	*35*	0.00015M	1.14	*1.06*
		3	0.97		0.34				1.02	
	VII	1	1.13		0.29				1.15	
		2	1.04	*1.03*	0.28	*0.27*	*28*	0.00023M	1.22	*1.14*
		3	0.92		0.25				1.04	

quantities of cyanide cause marked decrease or increase in the oxygen consumption. The results showed neither effect. The percentages in column 3 run slightly above the control but the difference is not significant, especially when it is considered that sets II, III and IV used more oxygen than set I in the control test also, column 1. This point has not been tested further as yet, but so far as these experiments go they do not give any evidence of a "stimulating effect" of very weak KCN upon the oxygen consumption of Planaria.

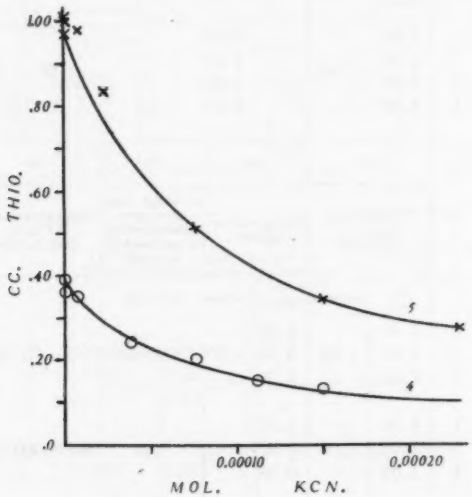

Fig. 1. Curves showing decrease in the oxygen consumption by Planaria agilis in KCN solutions of different concentration. Abscissas = molecular concentration of KCN. Ordinates = average oxygen consumption per bottle of worms, in cubic centimeters of thiosulphate. Curve *4* represents the data of table 4, column 2. Curve *5* represents the data of table 5, column 2.

The data of tables 4 and 5 have been plotted in figures 1 and 2. Figure 1 shows the average oxygen consumption per bottle in cubic centimeters of thiosulphate, plotted against the concentration of KCN. The curve marked *4* represents the data from table 4, and the curve marked *5* represents the data from table 5. Each point on curve *4* represents, therefore, the average of two determinations, and each point on curve *5* represents the average of three determinations. The two curves do not coincide because they represent the absolute amounts

of oxygen consumed by worms of different sizes, but it can be seen at a glance that they have the same general form. Figure 2 shows the average oxygen consumption in different KCN solutions, expressed in per cent of the control. As in figure 1, the curve marked *4* represents the data of table 4 and the curve marked *5* represents the data of table 5. Four things may be noted in figure 2: first, that the two curves almost coincide, showing that the two experiments, tables 4 and 5, give identical results; second, that the curves fall sharply; third, that they are not straight lines; and fourth, that they approach the

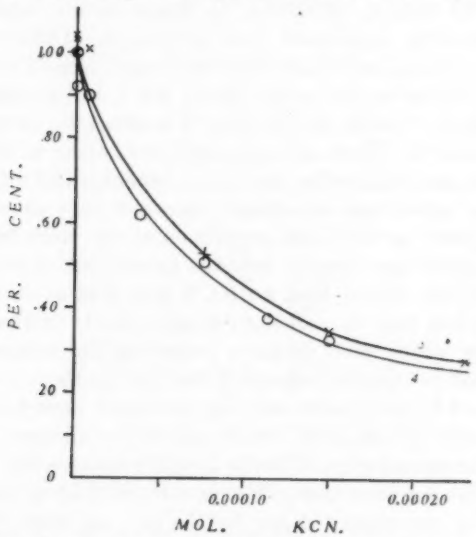

Fig. 2. Curves showing decrease in the rate of oxygen consumption by Planaria agilis in KCN solutions of different concentration. Abscissas = molecular concentration of KCN. Ordinates = rate of oxygen consumption in per cent of normal. Curve *4* represents the data of table 4, column 3. Curve *5* represents the data of table 5, column 3.

horizontal at about 20 per cent of the normal. We may conclude, therefore, that lower concentrations of KCN have proportionately greater effect upon the oxidations than more concentrated solutions; and second, that the oxidations cannot be reduced by cyanide below about 20 per cent of the normal. In other words, these results indicate that a residual oxidation amounting to about 20 per cent of the normal cannot be inhibited by KCN. No clear evidence is obtained from experiments 4 and 5 for a stimulation of oxidations in weak solutions of KCN.

OXYGEN CONSUMPTION BY PLANARIA IN KCN SOLUTIONS DURING TIME PERIODS OF DIFFERENT LENGTH

It is possible that the rate at which Planaria use oxygen in KCN solutions changes with time. Miss Hyman (7) reports finding such changes in the oxygen consumption by sponges for the first two or three half-hour periods. But when longer experimental periods are employed, as in the experiments reported in this paper, it seems improbable that the precise length of the period should alter the general character of the results. However, to make certain regarding this point, the following experiment was performed. Different sets of worms were tested for periods of different length from 2 to 36 hours. All sets were started at the same time. Set I was stopped after 2 hours, set II after 4 hours, set III after 8 hours, etc., as can be seen in column 1, table 6. Each set comprised two bottles of worms used as a control set and two bottles used as an experimental set.

All sets were tested first in aerated tap water as a general control for the second test, in which the experimental sets were treated with KCN. This preliminary control test was carried out in every respect like the second test except that no KCN was present in any of the bottles. Attention may be called to several points in the results of the control test which give evidence regarding the accuracy of the methods. It will be noted in column 2 that for the short periods more worms were used in each bottle and for the longer periods fewer individuals were used. Column 3, which states the amount of oxygen absorbed by the worms in each bottle in cubic centimeters of thiosulphate, shows that not more than the equivalent of 2.20 cc. thiosulphate was absorbed by the worms in any bottle, i.e., not more than about half of the amount of oxygen available in each bottle at the beginning of the period, 4.21 cc. thiosulphate. The worms of the longer experimental periods were not subjected, therefore, to oxygen starvation. In the next place, the degree of uniformity among the individual determinations is shown in column 4. In this column the oxygen consumption has been reduced to the common basis of the number of cubic centimeters of oxygen gas at normal temperature and pressure absorbed per 10 worms per 24 hours. The individual determinations ranged from 4.02 to 4.50 cc. of oxygen, with the exception of one determination, set Vb, bottle 1, which was as low as 3.38 cc. oxygen. An examination of further records of this particular lot of worms is instructive. Table 8 gives the oxygen consumption by the worms in each

TABLE 6

Showing that Planaria agilis in a KCN solution maintains approximately the same rate of oxygen consumption during time periods of very different length. Worms 15 to 16 mm. in length and very broad; without food for 18 days previous to the experiment except feedings on the 5th and 6th days before the experiment. Concentration of KCN = 0.00003 molecular. All bottles started at the same time and analyzed after periods of different length, as noted. Temperature 1.94° to 20.0°C. All worms alive at the end of the experiment. 1 cc. thiosulphate = 0.1747 cc. oxygen at N. T. P.

PART	SET		BOTTLE			FIRST TEST (CONTROL)				SECOND TEST			
						Blanks				Blanks			
						cc. thio		average		cc. thio	average		
A	Blanks (No worms present)		1			4.18		4.21		4.05	4.08	No KCN	
			2			4.24				4.10		No KCN	
			3			4.23				4.10		KCN	
			4			4.18				4.08		KCN	
				(1)	(2)	(3)		(4)	(5)	(6)		(7)	(8)
				Length of test period	Number of worms in each bottle	Oxygen consumed (Control)		Oxygen consumption per 10 worms per 24 hours	Average rate of oxygen consumption in per cent of normal	Oxygen consumed		Average rate of oxygen consumption in per cent of normal	KCN or no KCN
				hrs.		cc. thio	average	cc. oxygen	per cent	cc. thio	average	per cent	
B	I	a	1	2	20	0.72	0.73	4.32	(Normal)	0.71	0.72	(Normal)	No KCN
			2	2	20	0.74		4.44		0.73			No KCN
		b	1	2	20	0.71	0.73	4.26	100	0.53	0.47	65	KCN
			2	2	20	0.75		4.50		0.41			
	II	a	1	4	20	1.42	1.38	4.26	(Normal)	1.28	1.29	(Normal)	No KCN
			2	4	20	1.34		4.02		1.30			
		b	1	4	20	1.37	1.42	4.11	103	0.84	0.84	65	KCN
			2	4	20	1.46		4.38		0.84			
	III	a	1	8	15	2.11	2.11	4.22	(Normal)	2.02	2.01	(Normal)	No KCN
			2	8	15	2.10		4.20		1.99			
		b	1	8	15	2.13	2.09	4.26	99	1.20	1.17	58	KCN
			2	8	15	2.05		4.10		1.13			

TABLE 6—*Continued*

PART	SET		BOTTLE	(1) Length of test period	(2) Number of worms in each bottle	(3) Oxygen consumed (Control)		(4) Oxygen consumption per 10 worms per 24 hours	(5) Average rate of oxygen consumption in per cent of normal	(6) Oxygen consumed		(7) Average rate of oxygen consumption in per cent of normal	(8) KCN or no KCN
				hrs.		*cc. thio*	*average*	*cc. oxygen*	*per cent*	*cc. thio*	*average*	*per cent*	
B	IV	a	1	12	10	2.06	*2.08*	4.12	(Normal)	1.98	*2.05*	(Normal)	No KCN
			2	12	10	2.10		4.20		2.11			
		b	1	12	10	2.03	*2.06*	4.06	*99*	1.10	*1.12*	*55*	KCN
			2	12	10	2.08		4.16		1.13			
	V	a	1	24	5	2.20	*2.18*	4.40	(Normal)	2.08	*2.07*	(Normal)	No KCN
			2	24	5	2.15		4.30		2.05			
		b	1	24	5	1.69	*1.87*	3.38	*86*	0.92	*1.02*	*49**	KCN
			2	24	5	2.04		4.08		1.12			
	VI	a	1	36	3	1.88	*1.95*	4.16	(Normal)	1.73	*1.81*	(Normal)	No KCN
			2	36	3	2.02		4.49		1.88			
		b	1	36	3	1.97	*2.00*	4.38	*103*	1.08	*1.09*	*60*	KCN
			2	36	3	2.02		4.49		1.10			

* Corrected to 57 per cent, cf. text page 111.

bottle of sets Va and Vb for five successive tests. In every test this particular lot of worms, set Vb 1, absorbed much less oxygen than any other lot. Evidently this lot of worms was not equivalent to the others in its normal capacity for absorbing oxygen and the low value of 3.38 cc. in table 6, column 4, set Vb 1, was due to irregularity of material rather than to any error in experimental measurement. Part of the variation between the other determinations also may be due to irregularities of material since the worms were selected for uniformity of size without measurement of their actual weights. It may be noted that column 4, table 6, shows no appreciable difference between the normal rate of oxygen absorption during short and longer periods of time. This is further evidence of the reliability of the experimental methods.

The amount of oxygen absorbed by the worms in each bottle during the second test is given in cubic centimeters of thiosulphate in column 6. In this test the experimental worms were treated with 0.00003 molecular KCN. This concentration was chosen because it has been found to cause a reduction in oxygen consumption which is intermediate between the maximum and minimum effects of cyanide. The oxygen consumption by worms in this solution during each of the different periods of time, from two to thirty-six hours, was compared with that of the corresponding untreated worms. For example, the average oxygen consumption by the control set, Ia, which were in pure water for 2 hours, was 0.72 cc. thiosulphate, column 6. The average oxygen consumption by the experimental set, Ib, which were in cyanide for the same period of 2 hours, was 0.47 cc. thiosulphate or 65 per cent of the normal. Similarly the average oxygen consumption of the other control sets: IIa, IIIa, IVa, Va, and VIa, column 6, were each taken as normal or 100 per cent, column 7, with which were compared the average oxygen consumption of the corresponding experimental sets: IIb, IIIb, IVb, Vb, and VIb. Similar calculations for the first test, column 5, showed that the experimental sets were equivalent to the control sets in every case excepting set V, which has been discussed above. Column 7 shows, then, that the cyanide-treated worms in the second test used about 60 per cent of the normal amount of oxygen. As explained above, the worms in set Vb 1 were not equivalent to those in other bottles. When this fact is taken into account, the 49 per cent in column 7, set Vb, becomes 57 per cent.

In this experiment the rate of oxygen consumption in cyanide solution was practically the same during periods of different length from two to thirty-six hours. The inhibitory action of KCN on oxidations in Planaria was immediate or very rapid, therefore, and it did not change with time, at least within the limits of the experiment. There was no evidence of an initial stimulating effect of cyanide on the oxidations.

REVERSIBILITY OF THE ACTION OF KCN ON PLANARIA

Potassium cyanide is sometimes classed among the anesthetics. One of the characteristics of the action of the anesthetics is their reversibility, i.e., the fact that if the anesthetic has not been given in too strong concentration and has not acted for too long a time, the organism recovers from its effect rapidly and completely after removal

from its action. Several experiments demonstrated that the action of KCN on the oxygen absorption by Planaria is reversible.

Table 5, column 5, shows the complete recovery of the original rate of oxidation by Planaria after their removal from solutions of cyanide in which their oxidations were only 28 to 53 per cent of the normal.

TABLE 7

Showing reversibility of the effect of KCN on the oxygen consumption by Planaria agilis. Worms 15 to 16 mm. in length and very broad; without food for 18 days preceding the experiment except feedings on the 5th and 6th days previous to experiment; 20 individuals in each bottle. Each test period of 4 hours duration. Concentration of KCN solution = 0.00003 molecular. After worms were removed from the KCN of the 2nd test they were washed repeatedly in tap water and set for the 3rd test within 30 minutes. Data of the 1st and 2nd tests included also in table 6. Temperature 19.4° to 20.0°C. 1 cc. thiosulphate = 0.1747 cc. oxygen at N. T. P.

PART	SET	BOTTLE	FIRST TEST (CONTROL)		SECOND TEST			THIRD TEST		FOURTH TEST	
			Blanks		Blanks			Blanks		Blanks	
			cc. thio	*average*	*cc. thio*	*average*		*cc. thio*	*average*	*cc. thio*	*average*
A	Blanks (No worms present)	1	4.18		4.05		No KCN	4.31		4.36	
		2	4.24	*4.21*	4.10	*4.08*	No KCN	4.34	*4.34*	4.39	*4.37*
		3	4.23		4.10		KCN	4.38		4.40	
		4	4.18		4.08		KCN	4.32		4.34	
			(1)		(2)		(3)	(4)		(5)	
			Oxygen consumed		Oxygen consumed		KCN or no KCN	Oxygen consumed		Oxygen consumed	
			cc. thio	*average*	*cc. thio*	*average*		*cc. thio*	*average*	*cc. thio*	*average*
B	IIa	1	1.42	*1.38*	1.28	*1.29*	No KCN	1.30	*1.34*	1.40	*1.43*
		2	1.34		1.30			1.38		1.45	
	IIb	1	1.37	*1.42*	0.84	*0.84*	KCN	1.41	*1.43*	1.48	*1.47*
		2	1.46		0.84			1.44		1.46	
Per cent of normal . .				*103*		*65*			*107*		*103*

Twelve hours intervened between the second and third tests in this experiment so that the experiment gives no idea of the rapidity of the recovery of the normal rate, but it does show completeness of recovery.

Table 7 shows that the recovery is as rapid as is the action of the cyanide in reducing the oxidations. The first test, column 1, was the control test. The second test, column 2, showed a decrease in the

oxygen consumption to 65 per cent of the normal in 0.00003 molecular KCN. The third and fourth tests show complete recovery of the normal consumption in tap water. The third test followed the second with only thirty minutes interval. In this experiment the test periods were each of four hours' duration. The first and second tests of this

TABLE 8

Showing reversibility of the effect of KCN on the oxygen consumption by Planaria agilis. Worms 15 to 16 mm. in length and very broad; without food for 18 days preceding experiment except for feedings on the 5th and 6th days previous to the experiment; 5 individuals in each bottle. Concentration of KCN, 0.00003 molecular. After worms were removed from KCN of the 2nd test they were washed repeatedly in tap water and put into bottles for the 3rd test within 17 minutes. Temperature 19.4 to 20.0°C. 1 cc. thiosulphate = 0.1747 cc. oxygen at N. T. P. Data of 1st and 2nd tests included in table 6.

PART	SET	BOTTLE	FIRST TEST		SECOND TEST			THIRD TEST		FOURTH TEST		FIFTH TEST	
			Blanks		Blanks			Blanks		Blanks		Blanks	
			cc. thio	average	cc. thio	average	KCN or no KCN	cc. thio	average	cc. thio	average	cc. thio	average
A	Blanks (No worms present)	1	4.18		4.05		No KCN	4.23		4.35		4.17	
		2	4.24	*4.21*	4.10	*4.08*	No KCN	4.30	*4.37*	4.38	*4.36*	4.18	*4.18*
		3	4.23		4.10		KCN	4.31		4.39		4.20	
		4	4.18		4.08		KCN	4.24		4.32		4.16	
			(1)		(2)		(3)	(4)		(5)		(6)	
			Oxygen consumed in 24 hours		Oxygen consumed in 24 hours		KCN or no KCN	Oxygen consumed in 6 hours		Oxygen consumed in 6 hours		Oxygen consumed in 24.6 hours	
			cc. thio	average	cc. thio	average		cc. thio	average	cc. thio	average	cc. thio	average
B	Va	1	2.20		2.08		No KCN	0.63		0.59		2.00	
		2	2.15	*2.18*	2.05	*2.07*	No KCN	0.65	*0.64*	0.58	*0.59*	1.95	*1.98*
	Vb	1	1.69		0.92		KCN	0.52		0.49		1.52	
		2	2.04	*1.87*	1.12	*1.02*	KCN	0.67	*0.60*	0.56	*0.53*	1.89	*1.71*
Per cent of control				*86*		*49*			*94*		*90*		*86*

table are included also in table 6. After a short exposure, therefore, to cyanide which reduced the oxygen consumption 40 per cent, the consumption rose again to normal after removal from the cyanide solution, as rapidly as it fell in the cyanide.

Table 8 shows a similar rapid recovery of the normal oxygen consumption by worms after long exposure to cyanide solutions of the

same concentration. In the control test, test 1, set Vb, the experimental worms used 86 per cent as much oxygen as the control worms, test 1, set Va, so that this is the normal consumption of these worms. In the second test the worms in 0.00003 molecular KCN, column 2, set Vb, used 49 per cent as much oxygen as the control, column 2, set Va, i.e., they used 57 per cent as much as was normal for the experimental worms. In the third, fourth and fifth tests, the experimental worms recovered their normal consumption of oxygen. The first and second tests were each twenty-four hours in duration and the third and fourth were each six hours in duration. The interval between the second and third tests was only seventeen minutes. After exposure to the action of cyanide for twenty-four hours, therefore, the worms recovered so rapidly after removal from the cyanide solution that the normal rate was maintained for the first six hours. The first and second tests of this experiment are included also in table 6.

RELATION BETWEEN THE OXYGEN CONSUMPTION BY PLANARIA IN KCN AND LOCOMOTOR ACTIVITY

Since it has been demonstrated by numerous experiments that the oxygen consumption by Planaria falls very markedly in KCN solutions, the question arises whether this decrease can be accounted for by decreased locomotion in the cyanide solutions. The locomotion of Planaria can be stopped, as has been stated by Child (1, p. 161), by cutting off the head and this operation does not involve serious injury to the worm. To get a picture of how effective this measure is in stopping locomotion, the experiment recorded in table 9 was performed. Four bottles containing five worms each were placed in a darkened room where they could be observed very carefully without disturbing the worms. The first bottle contained normal worms; the second bottle, worms with the heads removed close behind the auricles; the third bottle, worms with the heads removed a short distance in front of the pharynx; and the fourth bottle contained worms in a 0.000076 molecular solution of KCN. All bottles were observed at frequent intervals during eight hours. At each observation the number of worms found in movement in each bottle was recorded. After the first few observations the positions of the different individuals were easily remembered and any worm which had changed position since the last observation was counted as in motion. This was very convenient in the case of the worms in the cyanide since they moved very

slowly. The table shows that the removal of the head stops movement immediately and almost completely. After the original disturbance the normal worms required some time before coming to rest, but once at rest they remained so for hours, if not disturbed. The

TABLE 9

Showing that removal of the head of Planaria agilis is more effective than 0.000076 molecular KCN in stopping locomotion. Worms 12 to 14 mm. in length; 5 individuals in each bottle. Worms of bottle I were normal. Bottle II, heads of worms removed a short distance posterior to auricles at 9:00 a.m. Bottle III, heads removed a short distance anterior to pharynx. Bottles I, II and III placed in dim light at 9:05 a.m. and not again disturbed. Bottle IV, worms placed in 0.000076 molecular KCN at 9:40 a.m.

A NUMBER OF INDIVIDUALS IN MOTION OR MOVED SINCE LAST OBSERVATION					A—*continued*					A—*continued*				
	I	II	III	IV		I	II	III	IV		I	II	III	IV
	Normal	Headless	Headless	KCN		Normal	Headless	Headless	KCN		Normal	Headless	Headless	KCN
9:10 a.m.	5	0	1		10:38 a.m.	2	0	0	4	1:30 p.m.	0	0	0	3
9:15 a.m.	5	0	0		10:45 a.m.	1	0	0	2	1:45 p.m.	0	0	0	3
9:20 a.m.	5	0	0		10:50 a.m.	1	0	0	2	2:05 p.m.	0	0	0	1
9:25 a.m.	4	0	0		10:55 a.m.	1	0	0	2	2:15 p.m.	0	0	0	1
9:30 a.m.	4	0	0		11:00 a.m.	2	0	0	3	2:30 p.m.	0	0	0	0
9:35 a.m.	3	0	0		11:05 a.m.	1	0	0	2	2:45 p.m.	0	0	0	1
9:40 a.m.	3	0	1		11:10 a.m.	1	0	0	2	3:00 p.m.	0	0	0	1
9:45 a.m.	3	0	0	5	11:15 a.m.	1	0	0	1	3:20 p.m.	0	0	0	1
9:50 a.m.	3	0	1	5	11:20 a.m.	1	0	0	2	3:45 p.m.	0	0	0	0
9:55 a.m.	4	0	1	5	11:25 a.m.	1	0	0	3	4:00 p.m.	0	0	0	0
10:00 a.m.	3	0	1	5	11:35 a.m.	1	0	0	2	4:15 p.m.	0	0	0	0
10:05 a.m.	1	0	1	5	11:45 a.m.	1	0	0	3	4:30 p.m.	0	0	0	2
10:10 a.m.	1	0	1	5	11:55 a.m.	0	0	0	3	4:50 p.m.	0	0	0	2
10:15 a.m.	1	0	0	5	12:05 p.m.	0	0	0	2	5:05 p.m.	0	0	0	2
10:20 a.m.	1	0	1	5	12:25 p.m.	0	0	0	4	5:25 p.m.	0	0	0	0
10:25 a.m.	1	0	0	5	1:00 p.m.	0	0	0	0					
10:30 a.m.	1	0	0	5	1:15 p.m.	0	0	0	3					

bottle of cyanide-treated worms was started thirty-five minutes later than the other bottles, but if the table is read with this is mind it will be seen that the worms in cyanide were in motion for even more of the time than the normal worms not in cyanide, although the rate of motion in cyanide was very slow. This experiment shows, there-

TABLE 10

Showing that KCN reduces the rate of oxygen consumption by Planaria agilis far more than does removal of the head. Worms 12 to 14 mm. in length; fed almost daily till the day before the experiment; 10 individuals in each bottle. Removal of heads performed within the half hour preceding the second test period. Each test period of 10 hours duration. Concentration of KCN, 0.000076 molecular. Temperature 19.6° to 19.7°C. Correction for 6 cc. of water displaced by rods and reagents: multiply by 1.048. 1 cc. thiosulphate = 0.1751 cc. oxygen at N. T. P.

PART	SET	BOTTLE	FIRST TEST (CONTROL)						SECOND TEST								
			Blanks						Blanks								
			cc. thio.		average				cc. thio.		average						
A	Blank (no worms present)	1	4.01						3.99								
		2	4.03						3.97								
		3	4.03						3.99								
		4	4.05		4.04				4.01		4.00						
		5	4.06						4.01								
		6	4.04						4.01								
			(1)		(2)	(3)			(4)		(5)	(6)			(7)	(8)	(9)
			Oxygen consumed		Weight of worms in each bottle	Oxygen consumption per gram per 24 hours			Oxygen consumed		Weight of worms in each bottle	Oxygen consumption per gram per 24 hours			Average rate of oxygen consumption in per cent of normal	Normal or headless	KCN or no KCN
			cc. thio.	average	grams	cc. oxygen	average		cc. thio.	average	grams	cc. oxygen	average		per cent		
B	I	1	0.53		0.0397	5.9			0.45		0.0396	5.0					
		2	0.54	0.54	0.0426	5.6	5.8		0.46	0.48	0.0421	4.8	5.2		(Normal)	Normal	No KCN
		3	0.54		0.0400	5.9			0.53		0.0394	5.9					

B	II	1	0.52	*0.52*	0.0430	5.3	*5.4*	0.21	*0.20*	0.0409	2.3	*2.2*	42	Normal	KCN
		2	0.55		0.0435	5.6		0.20		0.0420	2.1				
		3	0.49		0.0403	5.4		0.20		0.0389	2.3				
	III	1	0.57	*0.53*	0.0405	6.2	*5.9*	0.36	*0.35*	0.0377	4.2	*4.3*	83	Headless	No KCN
		2	0.52		0.0416	5.5		0.36		0.0360	4.4				
		3	0.50		0.0375	5.9		0.33		0.0331	4.4				
	IV	1	0.53	*0.51*	0.0411	5.7	*5.6*	0.15	*0.17*	0.0347	1.9	*2.1*	40	Headless	KCN
		2	0.50		0.0410	5.4		0.16		0.0353	2.0				
		3	0.49		0.0388	5.6		0.19		0.0329	2.5				
	V	1						0.20					Heads of Set III		No KCN
		2						0.21					Heads of Set IV		No KCN

fore, that the locomotion is stopped more completely by cutting off the head than by immersion in cyanide.

It remains, then, to determine the relative effects of cutting off the head and of immersion in cyanide solutions upon the oxygen consumption by Planaria. The experiment summarized in table 10 shows that 0.000076 molecular KCN reduced the oxygen consumption three times as much as cutting off the head. In other words, the cyanide had three times as much effect in inhibiting the oxidations in Planaria as could be attributed to loss of movement. Furthermore, there was still some movement in the cyanide solution..

. This experiment involves an element not included in other experiments reported in this paper. When the head of a worm is cut off a considerable amount of tissue is removed, so that the amount of oxygen consumed after such removal cannot be compared with that before the removal, unless account is taken of the loss of body weight. In this experiment, therefore, the set of worms used in each bottle was weighed after each experimental period. The methods of weighing will be described in a later paper. Table 10, part A, gives the analyses of the blanks as heretofore. Part B, column 1, gives the oxygen consumption by the worms in each bottle during the control test. Column 2 gives the weight of the worms in each bottle and column 3 gives the oxygen consumption by these worms in cubic centimeters of oxygen gas at normal temperature and pressure per gram weight of worms per 24 hours. For the second test, columns 4, 5 and 6 give the corresponding data. The worms of set I were control worms, uncut and not in cyanide. They consumed 5.2 cc. of oxygen per gram per 24 hours during the second test. Set II were uncut worms in 0.000076 molecular KCN and they consumed 2.2 cc. of oxygen per gram per 24 hours, i.e., 42 per cent of the control or normal. Set III were headless worms, not in cyanide, and they consumed 4.3 cc. of oxygen per gram per 24 hours, i.e., 83 per cent of normal, twice as much as the normal worms in cyanide. Set IV were headless worms in cyanide. These had the same rate of oxygen consumption as the normal worms in cyanide. It may be concluded, therefore, that after eliminating its effect upon movement, KCN reduces the rate of oxidations at least 50 per cent, and when it is considered that the concentration of cyanide used in this experiment did not cause as great a decrease of the oxidations as the stronger solutions used in previous experiments, it may be concluded that, apart from its effect upon locomotion, KCN can inhibit the oxidations more than 50 per cent.

One further factor remains undetermined in these experiments. That is the relation of the loss of muscle tone in cyanide solutions to the reduction of the rate of oxidations. Headless worms contract strongly and it may be that they as well as the normal worms consume oxygen in maintaining a muscle tone which is lost in cyanide solutions.

SUMMARY

1. It has been proved that Winkler's method as carried out in these experiments is sufficiently reliable for the quantitative measurement of the oxygen consumption by Planaria.

2. The oxygen consumption by Planaria can be reduced to 30 per cent of the normal rate by KCN in a concentration as low as 0.0002 molecular.

3. The amount of the reduction varies with the concentration of the cyanide, not directly but as a power function, lower concentrations causing proportionately greater reduction than higher concentrations.

4. The relation between the concentration of KCN and its inhibitory effect upon the rate of oxidation in Planaria agilis indicates that a residual oxidation amounting to about 20 per cent of the normal cannot be inhibited by KCN.

5. No evidence was obtained of a stimulation of oxidations in low concentrations of KCN.

6. The rate at which Planaria absorbed oxygen in a cyanide solution was found to be practically the same during periods of different length from two to thirty-six hours. The effect of cyanide is therefore almost immediate and does not change materially with time.

7. The action of KCN upon oxidations in Planaria agilis is reversible, worms recovering their normal level of oxidations rapidly and completely after removal from cyanide solutions.

8. The inhibition of oxidations in Planaria by KCN could be attributed in only small part to the cessation of movement which results from the anesthetic action of cyanide.

9. These experimental results are considered important in themselves. Questions of their theoretical interpretation in relation to the toxic action of KCN and to the use of cyanide as a measure of the rate of oxidations are left for later consideration.

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THE TREATMENT OF SEVERE AND PROGRESSIVE HEMORRHAGE BY INTRAVENOUS INJECTIONS

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It is generally conceded that transfusion with properly matched human blood is the best treatment for patients suffering from the effects of hemorrhage, when employed in conjunction with the usual measures of heat, rest, etc. The difficulty of making careful transfusions in the advanced dressing stations and ambulances has made necessary resort to infusions with solutions of sodium chloride, bicarbonate, gelatin, gum arabic and so on. The cases reaching large hospitals in the rear, where there would be more time and better facilities for transfusions, are rarely in a condition of shock. During a six months' period in a hospital for French wounded in Paris, the author saw no patient admitted in a condition of shock.

The experiments reported here were carried out at the suggestion of Dr. W. H. Howell, as part of the work being done under the supervision of the Committee on Shock of the Medical Division of the National Research Council.

In all cases dogs were used under ether anesthesia preceded by a grain of morphia. As a rule no ether was given during the period of low blood pressure. The anesthetic was administered from an ether bottle by means of a rubber tube passed through the larynx into the trachea. Aseptic precautions were observed throughout. One of the carotid arteries was connected with a mercury manometer so that the arterial blood pressure might be observed throughout the experiment and recorded on a smoked drum from time to time. A mean between systolic and diastolic pressure was used in the tabulation of results. The right femoral artery was connected by a cannula with a long rubber tube through which the animal could be bled at desired intervals. Washout cannulas were employed both in the carotid and femoral arteries so that the end of the artery, the cannulas and tubes could be washed clear with 2 per cent sodium citrate solution.

In the case of the femoral artery, the blood was washed out after each bleeding so that no difficulty was encountered from clotting. A cannula in the femoral vein was connected with tubing for infusions and for the determination of venous pressure.

For the arterial blood pressure, an arbitrary level was chosen (40 mm. Hg. in most cases). By successive bleedings the pressure was brought down to the chosen level and kept there for varying lengths of time. It happened in some cases that the pressure tended to fall below 40 mm. Hg. down to 35 or 30 mm. before the set time had elapsed. In such cases the pressure was maintained by infusions of warm salt solution. At the end of the period the pressure was raised by infusion of one of the solutions to be tested. The course of the blood pressure was then observed for several hours and the wounds closed. If the animal survived, his condition was observed for a week.

The solutions used, which were infused at about 38°C., were *a*, solution of sodium chloride 0.9 per cent; *b*, gum-bicarbonate solution according to the formula formerly recommended by the British Shock Committee (1) i.e., 6 per cent gum acacia and 2 per cent sodium bicarbonate. The two solutions, gum 9 per cent and bicarbonate 6 per cent, were sterilized separately in the autoclave and combined in the proportion of 2 to 1 just before infusion. The gum was made up in hot tap water, allowed to stand 24 hours, strained through flannel and filtered under pressure through a Buchner funnel. In the sterilization it was necessary to guard carefully against escape of gas from the bicarbonate by using strong air-tight bottles. When gas had escaped there appeared a white granular precipitate on combining the two solutions preparatory to use. *c*, Gum glucose solution as recommended by Erlanger (2). In these experiments the solution used contained 6 per cent gum acacia and 6 per cent dextrose. The amount of infusion, as a rule, was equal to the amount of blood withdrawn from the animal. The total volume of blood was calculated to be 5 per cent of the animal's weight, although it will be noted that two animals survived the loss of 101 per cent of the total thus calculated.

Much of the previous work on intravenous infusions has been done by relieving animals of a certain per cent of their calculated blood volume and following this immediately by saline or other injection. It seemed that such animals are not strictly comparable to patients who have lost blood and then suffer from the effects of the anemia and acidosis attendant upon continued low blood pressure; that is, patients who have entered into a state of shock. Also, the ability of different

animals of the same species to withstand the same per cent of blood loss appears to vary greatly. It was therefore determined to bleed the animals until the arterial pressure reached 40 mm. Hg. and to maintain this pressure as nearly as possible by repeated hemorrhages or, if necessary, small saline infusions. At the end of this period of continued low blood pressure the solution to be tested was infused into the femoral vein.

Three animals were given injections of the sodium chloride solution after their arterial pressure had been about 40 mm. Hg. for less than forty-six minutes. All three recovered although they had lost 79 per cent, 69 per cent and 85.5 per cent respectively of their total calculated blood volume. It was thought that by exposing the animal to a longer period of low pressure a time would be found at which infusion of salt solution alone would not suffice to save it and that this period might then be used as a standard in testing other solutions. With this thought in mind the period of low blood pressure was increased up to seventy-five minutes at 40 mm. pressure and up to three and one-half hours at 50 mm. Of three dogs kept at 50 mm. pressure for two and one-half hours and infused with salt solution, two survived (experiments 22, 23, 24). Of two, kept for three and one-half hours at 50 mm. pressure, and then treated with salt solution, one survived (experiments 25, 26). The minimum time at which all animals would die when infused with salt solution was not determined, but a number of animals were subjected to the same treatment, along the lines stated above, and infused with one of the three solutions.

Selecting all the experiments in which the blood pressure was kept at 40 mm. for more than sixty minutes, we derive table 1. The number of experiments is rather small for drawing conclusions, but treatment with saline appears as successful as that with the other two solutions.

If one compares the results of the infusions according to the percentage of blood lost in each case table 2 is obtained.

It is difficult to draw conclusions as to the efficacy of the solutions employed from the above table also but it does show very plainly that large quantities of blood may be lost, if only it be replaced by an intravenous injection and the pressure raised thereby. If the adequate injection be delayed too long there comes a time when the organism will no longer respond, the pressure falls and respiratory failure supervenes. This period varies greatly in different animals. In experiment 27, 86 per cent of the total calculated blood was lost. Thirty-

one minutes after the first bleeding the pressure had fallen to 26 mm. Hg. and 63 cc. saline solution were sufficient to raise it to 50 mm. After eighty-two minutes 52 cc. saline were sufficient to raise the pressure, which had again fallen, from 30 mm. up to 45 mm. Hg. At one hundred and twenty minutes 70 cc. saline raised the pressure from 27 to 44 mm.; one hundred and sixty-two minutes after the initial bleed-

TABLE 1

AT END OF LOW BLOOD PRESSURE TREATED WITH:	NUMBER OF EXPERIMENT	PRESSURE MAINTAINED	DURATION OF LOW PRESSURE	RESULT	BLOOD LOST IN PER CENT OF THE TOTAL CALCULATED VOLUME
			minutes		*per cent*
*Salt solution	5	40 mm. Hg.	60	Died	90
Salt solution	6	40 mm. Hg.	64	Lived	93
Salt solution	9	40 mm. Hg.	75	Lived 66⅔ per cent survival	94.5
Gum-bicarbonate solution	10	40 mm. Hg.	60	Lived	101
Gum-bicarbonate solution	14	40 mm. Hg.	62	Lived	101
Gum-bicarbonate solution	12	40 mm. Hg.	75	Died	90
Gum-bicarbonate solution	11	40 mm. Hg.	89	Died 50 per cent survival	101
Gum-glucose solution	17	40 mm. Hg.	62	Died	90
Gum-glucose solution	18	40 mm. Hg.	62	Died	83
Gum-glucose solution	16	40 mm. Hg.	63	Lived	87
Gum-glucose solution	15	40 mm. Hg.	70	Died 25 per cent survival	83

**Note.* No animal, treated with any infusion, lived after having lost more than 101 per cent of his total calculated blood volume. Hence, for purposes of comparison, experiments 2 and 7, where the blood loss was above that figure, are not included here.

ing 88 cc. were required to raise the pressure from 24 to 38 mm. At two hundred and ten minutes the pressure dropped rapidly to 12 mm., and injection of gum-bicarbonate solution caused only an increased heart rate but did not prevent the developing respiratory failure nor influence the progressive fall of blood pressure to zero.

While the animal has this low blood pressure, there results, as has been emphasized by Cannon (3), a condition of acidosis. This acidosis

develops gradually as shown in experiment 13 (see fig. 1) in which the pressure was held at about 40 mm. Hg. for sixty-eight minutes. Determinations were made upon blood specimens with the Van Slyke apparatus:

Alkaline reserve before 1st bleeding..... 45.068
Alkaline reserve 3 minutes after 1st bleeding..... 38.429
Alkaline reserve 32 minutes after 1st bleeding..... 18.812
Alkaline reserve 61 minutes after 1st bleeding..... 13.078

TABLE 2

BLOOD LOST IN PER CENT OF TOTAL CALCULATED VOLUME	SOLUTION FOR INTRAVENOUS INFUSION	RESULT
per cent		
69.0	Saline	Lived
71.0	Saline	Lived
74.0	Saline	Lived
75.0	Saline	Died
78.0	Saline	Lived
79.0	Saline	Lived
81.0	Saline	Lived
82.0	Saline	Died
83.0	Gum-glucose	Died
83.0	Gum-glucose	Died
85.5	Saline	Lived
87.0	Gum-glucose	Lived
90.0	Saline	Died
90.0	Gum-glucose	Died
90.0	Gum-bicarbonate	Died
91.0	Saline	Lived
93.0	Saline	Lived
94.5	Saline	Lived
101	Gum-bicarbonate	Lived
101	Gum-bicarbonate	Died
101	Gum-bicarbonate	Lived
102	Saline	Died
109	Saline	Died
110	Saline	Died

At the time of the last determination the pressure was 26 mm. Hg. Eight minutes later the animal was dead in spite of intravenous injections with saline and gum-bicarbonate solutions.

In experiment 14 the dog survived a reduction of blood pressure to 40 mm. for sixty-two minutes; at the end of which time gum-bicarbonate solution was injected. The alkaline reserve varied as follows:

Alkaline reserve before 1st bleeding..... 38.221
Alkaline reserve 24 minutes after 1st bleeding..... 25.274
Alkaline reserve 61 minutes after 1st bleeding..... 20.579

It will be noted that in the fatal case the acidosis was much more rapidly developed.

For the sake of comparing the result of treatment by the three solutions, all experiments in which the alkaline reserve was determined are tabulated.

TABLE 3

TREATED WITH SALINE SOLUTION			GUM-BICARBONATE SOLUTION			GUM-GLUCOSE SOLUTION		
Number	Alkaline reserve before injection	Result	Number	Alkaline reserve before injection	Result	Number	Alkaline reserve before injection	Result
24	22.900	Lived	12	24.057	Died	16	20.239	Lived
22	22.322	Lived	14	20.579	Lived	17	19.222	Died
21	17.517	Lived	28	16.749	Lived	18	18.345	Died
25	13.500	Died	13	13.078	Died	15	15.105	Died
23	12.812	Died						

With the exception of no. 12, in which there was a fatal outcome with a comparatively high alkaline reserve, it will be noted that all deaths occurred in animals whose reserve was low. If the degree of shock be measured by the amount of acidosis, gum-glucose solution appears the least useful while gum-sodium-bicarbonate solution appears to be no more efficacious as an infusion than saline.

In a number of the experiments a venous manometer was used to measure the height of water pressure necessary to cause a flow of saline solution in the femoral vein toward the auricles. In every case zero for these readings was the level of the femoral vein, this point being accurately determined by breaking the connection of tubing and venous cannula and allowing the liquid in the pressure column to fall to the level of the vein. With this point as zero, readings of venous pressure were taken at intervals of five to fifteen minutes. Whenever the pulse and respiration caused a corresponding rise and fall in the pressure column, while a determination was being made, the lowest point to which the pressure fell was recorded.

In all fatal cases observed the continued fall of arterial pressure was accompanied not by a fall of venous blood pressure but by a rise. In every case where a fall of arterial pressure was accompanied by a

rise of venous pressure for twenty minutes or more the outcome was fatal regardless of treatment. Figures 1, 2 and 3 will serve to illustrate this point. In eleven experiments the venous pressure was recorded at frequent intervals. Of the animals thus used seven died. In

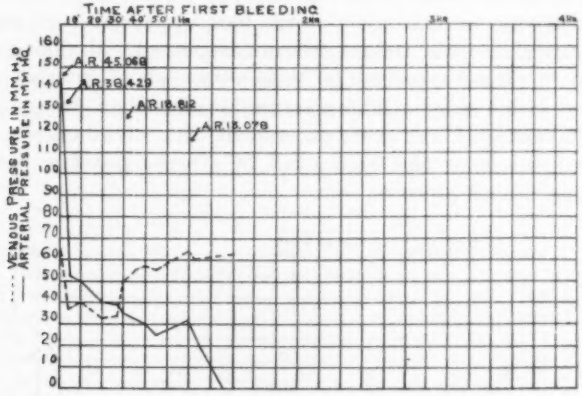

Fig. 1. Experiment 13; 100 per cent of total calculated blood volume removed. After 25 minutes the venous pressure rose steadily, the last reading being taken just before death. The corresponding increase of acidosis is measured by the diminishing alkaline reserve (A. R.) S-1 Infusion of 200 cc. normal saline.

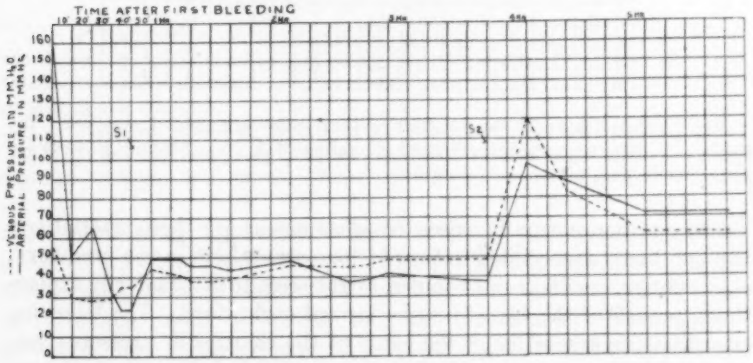

Fig. 2. Experiment 26; 71 per cent of the total calculated blood volume removed. In this case the dog recovered completely and there was no rise of venous pressure independent of arterial pressure. Infusions of: S-1, 68 cc. saline solution; 438 cc. saline solution.

every fatal case except one there was a period of twenty minutes or more before the infusion when the arterial pressure was falling and the venous pressure rising. The exception, experiment 18, had arterial and venous pressures which corresponded closely throughout. This animal, unlike the others, lived through the first twenty-four hours to succumb on the second day. Of the four which survived none had any considerable rise of venous pressure associated with falling arterial pressure. In these experiments, then, every fall of arterial pressure associated with a rising venous pressure for more than twenty minutes had a fatal outcome, and every experiment with a fatal outcome inside of twenty-four hours had such a fall of arterial pressure associated with rising venous pressure.

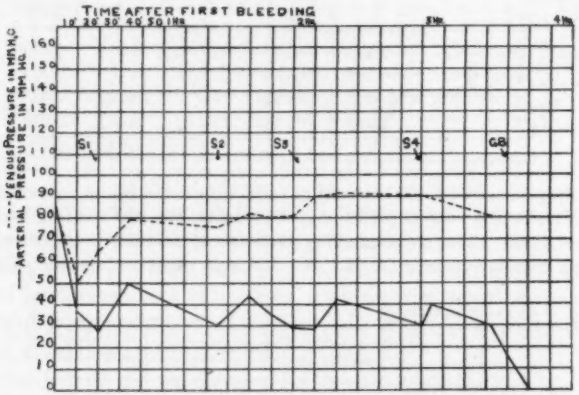

Fig. 3. Experiment 27; 86 per cent of total calculated blood removed. Infusions of: S–1, 62 cc. saline; S–2, 52 cc. saline; S–3, 70 cc. saline; S–4, 88 cc. saline; G-B, gum bicarbonate solution.

The question naturally arises: by what means are these arterial and venous changes associated? Loss of tone somewhere in the periphery, e.g., arteries, capillaries or venules, might seem to satisfactorily explain the approach of the two pressures toward each other. The following experiment is suggestive of the cause of the changes in pressures (see fig. 5). A dog was prepared so that the arterial and venous pressures could be recorded in the right femoral artery and vein. One carotid was cannulated so that blood could be drawn off. Cannulas were placed in a branch of the left femoral artery and of the left femoral

vein so that, on clamping the main artery and vein proximally, the vascular bed of the leg might be perfused with normal salt solution under a constant pressure and the amount of fluid passing through the leg in a given period measured. Then by clamping the cannulated branches and removing the clamps from the main vessels the leg could be again restored to the general circulation. The animal was bled, as in the previous experiments, so as to maintain the blood pressure at about 40 mm. Hg. Before the bleeding, 23.5 cc. of saline were perfused through the leg in one minute under a pressure equal to that in

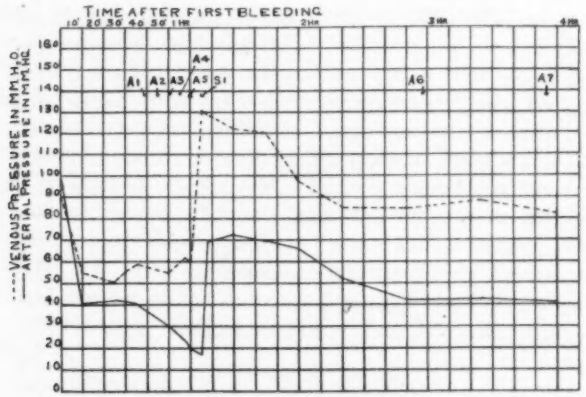

Fig. 4. Ninety-four per cent of total calculated blood volume lost. Low blood pressure maintained for one hour. Death occurred four hours after the infusion of saline solution. Atropine was injected intravenously with 1–4 cc. of normal salt solution.

A–1, 0.001 gm. atropine; A–2, 0.001 gm. atropine; A–3, 0.001 gm. atropine; A–4, 0.002 gm. atropine; A–5, 0.005 gm. atropine; A–6, 0.002 gm. atropine; A–7, 0.002 gm. atropine. S–1, 432 cc. normal salt solution.

the femoral artery of the other leg. After six minutes of low blood pressure, only 21.5 cc. of saline could be perfused in one minute under the same pressure, showing that there had been an increase in peripheral vascular tone. Following the first bleeding the venous pressure had fallen with the arterial pressure (fig. 5). After seventy-six minutes of low blood pressure, 28 cc. could be perfused. There had evidently been a loss of tone in the peripheral vessels since the last perfusion and reference to figure 5 makes it evident that the venous pressure had risen while the arterial pressure had fallen in that in-

terval. One hundred and thirty-two minutes after the first bleeding 36.5 cc. were perfused. The loss of vascular tone which is here indicated by the increased rate of flow through a peripheral vascular bed under constant pressure conditions might account for both falling arterial pressure and rising venous pressure. The data at hand throw no light on the condition of the heart, a progressive enfeeblement of which would contribute to the pressure changes observed. In this same experiment (no. 30), the pressure fell progressively to 4 mm. Hg., when respiratory failure supervened. At this point warm, normal

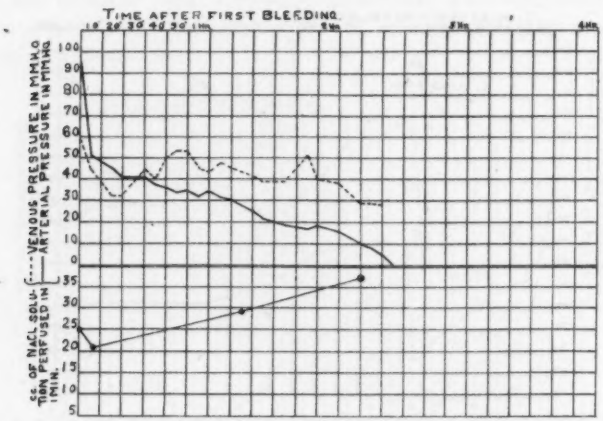

Fig. 5. Perfusion experiment. Blood loss was 61 per cent of the total calculated blood volume plus the unknown amount of blood washed out each time the hind limb was perfused. The vascular bed of this hind limb formed part of the general circulation except for four brief intervals when it was perfused with normal saline solution.

saline solution was injected into a vein and the heart, which had almost stopped, began to beat more forcibly. Its rhythm and amplitude of contraction, as well as may be estimated by the mercurial manometer, came back to what they had been at the beginning of the experiment and the pressure rose to 8 mm. Hg. for a few minutes. This would seem to indicate that not cardiac failure, but failure of venous return is the primary factor in the fatal result. The blood is evidently lost or pooled in the relaxed peripheral vessels.

Reid Hunt (4) in working on the action of acetyl choline as a vasodilator and depressor of blood pressure has shown that everywhere its

action is opposed by that of atropine. In view of the presence of choline as one of the products of metabolism it seemed advisable to inquire into the effect of atropine upon an animal when in a state of low blood pressure following hemorrhage (see fig. 4). The immediate effect of atropine upon the arterial pressure after each injection was in every case a very slight rise followed by a somewhat greater fall. The dog was infused with normal saline solution after the arterial pressure had been about 40 mm. Hg. for sixty-five minutes. The pressure rose to 73 mm. after the infusion but it was not necessary to readminister ether as it had been in most experiments. The pupils were dilated, the corneal reflexes present. Faradic stimulation of the central end of one vagus,—the other being intact—caused a period of apnoea but had no effect upon the heart rate. The animal died four hours after the saline injection, apparently deriving no benefit from the atropine.

In five experiments the saphenous nerve was stimulated at the end of the low blood pressure period, the stimulation being either electrical (induction shocks) or mechanical. In two, the respiration increased and the animal moved. In one, only the respiration was affected. Thirty minutes later this animal was dead. Bulbar reflexes seemed to be still active. In the two cases where a vasomotor reaction resulted some central control of the vasomotor system remained, although in one (exp. 10) the state of shock was so profound that the animal never roused from it.

Eight experiments similar to those described in this paper were carried out upon dogs that had been used for demonstration purposes, after two to six hours of ether anesthesia. The vagi had been cut and the saphenous nerve stimulated during the demonstration. In these animals a loss of 40 to 60 per cent of the calculated volume of blood brought about changes comparable to those we have noted above in animals losing 75 to 90 per cent.

SUMMARY AND CONCLUSIONS

The blood pressure of experimental animals was kept at a shock level for various lengths of time by progressive bleeding so that their condition resembled that which must frequently exist in wounded men at the front who have sustained severe and continued loss of blood. After analyzing the condition of these experimental animals according to the duration of low blood pressure, the amount of blood lost and the degree of acidosis resulting, I have not been able to show

that gum-sodium bicarbonate solution or gum-glucose solution is more efficacious in saving life than is an isotonic solution of sodium chloride.

By perfusing an isolated vascular bed it has been shown that following a large hemorrhage there is a primary constriction of the peripheral vessels. With continued low blood pressure this constriction passes off and dilatation supervenes. Associated with this dilatation the venous pressure rises while the arterial pressure is falling. All dogs in which the venous pressure rose while the arterial pressure was falling, for a period of twenty minutes or more, died regardless of treatment.

The effects of stimulation of the saphenous nerve while these animals were in a condition of profound shock indicated that the reflexes affecting respiration and blood pressure were not paralyzed, although this point deserves more careful investigation.

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